AN INVESTIGATION INTO THE MECHANISM OF PRODUCTION OF BLACKWATER

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(Being the Report of the Blackwater Fever Expedition to Nyasaland of the Liverpool School of Tropical Medicine, 1907-1909)

(Received for publication 6 *May,* 1909)

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INTRODUCTION

In the past the investigation of blackwater fever has been essentially clinical. The symptoms and course of the disease have been studied and, as regards aetiology, its relation to the administration of quinine has been established; but purely clinical observation has failed to explain the significance of its association with malaria, although its dependence upon malaria has been assumed by most writers. But little attention has been given to the mechanism of production of blackwater fever, although recent advances in experimental physiology and pathology, and above all in physical chemistry, have provided numerous methods applicable to research in this direction. In the investigation which we have made upon blackwater fever, we have endeavoured to trace out some of the internal processes, whose terminal event is the appearance of blackwater, believing that in this way many obscure points in connection with the causation and treatment of this condition would sooner or later be cleared up. In our work we have always attempted to give our observations a quantitative character, and to be as far as possible free from the limitation imposed by purely qualitative methods.

Some of the investigations which we have entered upon have necessarily been of a somewhat tentative character. Thus before the direct action of quinine upon the red blood cells in blackwater fever could be determined, it has been essential first of all to determine its action upon the red blood cells of healthy individuals. The next step undertaken was that of determining if haemolysins, present in the blood, played any part in the production of blackwater, such as is known to be the case in paroxysmal haemoglobinuria. A further point has been the investigation of the relationship of haemoglobinaemia to haemoglobinuria. Again, in studying the condition of the urine in blackwater fever, it has been found desirable to estimate, whenever possible, the amount of blood cells corresponding to the dissolved haemoglobin present in the urine, and for this purpose it has also been necessary to investigate quantitatively the action of urine upon haemoglobin. Further light upon the mechanism of production of blackwater fever has also been obtained by studying redwater and by experimentally producing haemoglobinuria in animals.[*]

The attacks of blackwater fever which we have investigated have been twenty in number, and were kindly brought under our observation by the Nyasaland Government medical staff and by the medical officers of the Shiré Highlands Railway Company.

Our thanks are also due to the Nyasaland Government for providing us with rooms at the Hospital at Blantyre.

Our report was completed in the Runcorn Research Laboratories of the Liverpool School of Tropical Medicine.

I. THE HAEMOLYSIS OF RED BLOOD CELLS BY QUININE AND ALSO BY ACID, ALKALI AND URINE.

a. The action of quinine, acid and alkali on healthy red blood cells.

1. Action of quinine bihydrochloride on red blood cells in varying concentrations.

In testing the haemolytic action of quinine[†] upon red blood cells the bihydrochloride was used because its greater solubility in water permitted the employment of a wider range of concentration than would have been possible had the monochloride or sulphate been chosen. In the first three series of experiments the action of the quinine salt was tested upon red blood cells freed from plasma, the method of procedure in these, and most of the succeeding experiments of the same type, being as follows :—

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[*] See Table of Contents, p. 1.

[†] The only work on this subject appears to be that of Nocht, *Über Schwarzwasserfieber*, Verhandlungen des deutschen Kolonialkongresses, 1905, who stated that 2 mgrm. of quinine in 5 c.cm. of a 10 per cent. dilution of defibrinated blood in a 0·9 per cent. sodium chloride solution was the maximum amount which could be added short of causing haemolysis.

Method.—About one cubic centimetre of blood was obtained by pricking the finger, previously washed and cleansed with absolute alcohol, and allowing blood to flow drop by drop into a small glass collecting tube containing a measured amount of a 1 per cent. solution of potassium oxalate (containing also 0·45 per cent. of sodium chloride, so as to be isotonic with the red cells), in the proportion of one part of the latter to from four to ten parts of blood. The mixture thus obtained was next sucked up into a graduated 2 c.cm. pipette and its volume noted. The percentage (by volume) of red cells contained in the mixture was then determined by means of a haemocrit. The mixture, or a measured fraction of it, was next added to about 10 c.cm. of 0·9 per cent. solution of sodium chloride and centrifugalised until the red cells were completely precipitated. The supernatant fluid was then pipetted off and as much of the 0·9 per cent. solution of sodium chloride added as was required to make up a 2·5 per cent. emulsion of red cells. In many of the experiments a portion of this emulsion was laked by the addition of distilled water, and the amount of haemoglobin it contained determined by means of a haemoglobinometer reading, the absolute values of the scale of the instrument having previously been determined in terms of the red cells in the moist condition of one of us, taken as a standard. In this way the value of different blood emulsions made up to 2·5 per cent. of wet red cells can be referred, as regards their haemoglobin content, to the same standard. It may be observed that sometimes, as for example in cases of malaria, as will be seen later (cp. Table 24), this is absolutely necessary if different experimental results are to be compared.

The red blood cell emulsion having been prepared as above described measured amounts were then added to a series of test-tubes containing a quinine solution isotonic with blood plasma and, if necessary, also 0·9 per cent. of sodium chloride, so as to make up a diminishing series of concentrations of quinine salt, while at the same time the percentage of wet red cells in the series progressively increased (Table 1). In this way the point was determined at which the red blood cells present were completely haemolysed at the end of the period of experiment, namely, three hours, the temperature of experiment being 37° C. In Table 1, only the concentration of quinine salt and the ratio of the weight of wet red cells to the weight of quinine present are given, the actual quantities of red blood cell emulsion, quinine solution and of salt solution, which were employed, being omitted so as not to overburden the Table with such details of experiment as are best arranged in accordance with the actual conditions of working which individual experimenters may regard as most convenient. In some of the later experiments, however, the concentration of the solutions employed and the amounts of each used are given (Table 24); from these the reader will readily devise convenient concentrations and quantities to employ in those experiments in which these details are omitted.

TABLE 1. Haemolysis of red blood cells by quinine bihydrochloride dissolved in 0·9 per cent. NaCl solution. Duration of experiment three hours. Temperature 37° C.

No. of Experiment.	COMPOSITION OF MIXTURE OF RED BLOOD CELLS AND QUININE SOLUTION					
	Quinine bihydrochloride	0·080 %	0·065 %	0·054 %	0·045 %	0·038 %
	$\frac{\text{Weight of wet red blood cells}}{\text{Weight of quinine salt}}$	$\frac{5 \cdot 2}{1}$	$\frac{6 \cdot 5}{1}$	$\frac{8 \cdot 5}{1}$	$\frac{10 \cdot 4}{1}$	$\frac{13 \cdot 7}{1}$
1		Complete	Complete	Complete	Complete	Partial
2		Complete	Complete	Complete	Complete	Marked
3		Complete	Complete	Complete	Complete	Partial
4		Complete	Complete	Complete	Complete	Partial
5		Complete	Complete	Complete	Complete	Partial

During the course of the experiments the tubes containing the mixtures of red blood cell emulsion and quinine solution were stirred with a glass rod every fifteen minutes. In the tubes in which complete haemolysis appeared to have occurred, a microscopical examination of the deposit after centrifugalisation was made in order to make certain that this was the case.

In all these experiments the glass tubes and pipettes used were kept scrupulously clean. From time to time a search for bacteria was made at the close of the experiment, but uniformly with negative result. Actual sterilisation of the glass apparatus and of the solutions employed was carried out only in a few cases in which the time elapsing between the collection of blood and the termination of the experiment exceeded four hours.

On reference to Table 1 it will be seen that haemolysis was complete up to a concentration of 0·045 per cent. of quinine bihydrochloride, at which concentration the weight of red cells haemolysed was 10·4 times that of the quinine salt employed. When a concentration of 0·038 per cent. was employed, the weight of red cells being now 13·7 times that of the quinine salt present in solution, haemolysis was only partial, and on extending the series beyond the limit given in the Table, the red cells were found not to be appreciably affected. The transition point was sharply marked, though slight variations in the rate at which haemolysis proceeds were to be observed. As will appear later (cp. Table 18), in different samples of red blood cells, taken from the same (healthy) individual, similar variations, inconsiderable in extent, occur.

In the more concentrated solutions of quinine bihydrochloride haemolysis proceeds very rapidly. In the first two concentrations given in Table 1, haemolysis was complete within an hour, and in the third at the end of about two hours.

A second series of experiments (Table 2) was next carried out, in which the concentration of the quinine salt at the transition point was nearly four times that present at the transition point of the first series. In these, as in the former experiments, the transition point

TABLE 2. Haemolysis of red blood cells by quinine bihydrochloride, in nearly four times the concentration employed in Table 1. Duration of experiment three hours. Temperature 37° C.

No. of Experiment	COMPOSITION OF MIXTURE OF RED BLOOD CELLS AND QUININE SOLUTION					
	Quinine bihydrochloride	0·223 %	0·188 %	0·155 %	0·134 %	0·119 %
	Weight of wet red blood cells / Weight of quinine salt	10·4 / 1	13·0 / 1	15·6 / 1	18·2 / 1	20·8 / 1
1		Complete (Marked)*	Complete (Partial)	Marked (Slight)	Marked (Slight)	Slight (Slight)
2		Complete (Complete)	Complete (Marked)	Marked (Slight)	Slight (Slight)	Slight (Slight)
3		Complete (Complete)	Complete (Complete)	Complete (Slight)	Marked (Slight)	Slight (Slight)
4		Complete (Complete)	Complete (Complete)	Complete (Complete)	Marked (Slight)	Slight (Slight)
5		Complete (Complete)	Complete (Complete)	Complete (Marked)	Slight (Slight)	Slight (Slight)

* The results in brackets represent the action of quinine bihydrochloride when, instead of washed red cells, equivalent amounts of oxalated blood are used.

is well marked, and occurs when a concentration of quinine bihydrochloride amounting to 0·155 per cent. is reached. It will be noticed that in two members of the series, haemolysis, at the transition point, was nearly but not quite complete at the end of three hours at 37° C., the rate at which the reaction proceeded being in these two cases slightly slower than in the remaining members of the same group. In the succeeding concentration [0·134 per cent.] of quinine salt haemolysis is partial or slight, and in the remaining concentration (0·119 per cent.) but little haemolysis occurs. In these experiments it is seen that a given weight of quinine bihydrochloride does not haemolyse a fixed weight of red blood cells during the period of experiment (three hours), but that the maximum weight of red blood cells, which can be haemolysed, depends upon the concentration of the quinine salt, not upon its absolute amount.

A third series of experiments was now carried out with still higher concentrations of quinine salt (Table 3). Here, as before, the transition point was well marked. It was obtained when a concentration of quinine salt amounting to 0·635 per cent., i.e. about fourteen times that obtaining in the first series [0·045 per cent.] and nearly two and a half times that present in the second series [0·155 per cent.], was reached. The weight of the red blood cells haemolysed was 36·5 times that of the quinine salt present. As in the second series, so again here, the increase in the amount of red blood cells haemolysed is not in the same proportion as is that of quinine salt present, but is much smaller than the latter.

TABLE 3. Haemolysis of red blood cells by quinine bihydrochloride, in nearly fourteen times the concentration employed in Table 1. Duration of experiment three hours. Temperature 37° C.

No. of Experiment	COMPOSITION OF MIXTURE OF RED BLOOD CELLS AND QUININE SOLUTION.					
	Quinine hydrochloride	0·844 %	0·724 %	0·635 %	0·527 %	0·448 %
	Weight of wet red blood cells	22	29	36·4	46	57
	Weight of quinine salt	1	1	1	1	1
1		Complete	Complete	Complete	Partial	Slight
2		Complete	Complete	Marked	Partial	Slight
3		Complete	Complete	Complete	Marked	Slight
4		Complete	Complete	Complete	Partial	Slight
5		Complete	Complete	Complete	Partial	Slight

Up to the present only washed red blood cells, freed from plasma, have been considered. In Table 2, however, side by side with the results obtained with washed red cells, are given, in brackets, those obtained with the same amounts of red cells not freed from plasma. The haemolysis in the latter case is not so marked as in the former, the transition point being reached when a concentration of quinine bihydrochloride amounting to 0·188 per cent. is attained (as against 0·155 per cent. in the former series), and the weight of red blood cells haemolysed is thirteen times that of the quinine salt present (as against 15·6 in the former series), some of the quinine being apparently taken up by the plasma and thus prevented from acting upon the red blood cells. There is also noticeable, less regularity in this series, when compared with the former. This is perhaps attributable to diurnal variations in the composition of the plasma.

In Table 4 the action of quinine bihydrochloride upon red blood cells contained in more or less diluted oxalated plasma is shown. In these experiments the blood employed formed from 42 per cent. to 96 per cent. of the mixture. As in the preceding experiments the protective action of the plasma is well marked. Thus in Experiment 9, in which 84 per cent. of blood is present and the concentration of

TABLE 4. Haemolysis of red blood cells by quinine bihydrocholoride dissolved in the blood plasma. Temperature of experiment 37° C.

No. of Experiment	Source of blood	COMPOSITION OF MIXTURE OF OXALATED BLOOD AND SOLUTION OF QUININE BIHYDROCHLORIDE			Condition of mixture at end of experiment
		blood	Q.2HCl	$\frac{\text{weight of wet red cell}}{\text{weight of Q.2HCl}}$	
1	Human	96 %	0·21 %	195 : 1	Plasma contained 0·6 % of dissolved haemoglobin at the end of three hours.
2	,,	92 %	0·41 %	96 : 1	,, 0·4 % ,,
3	,,	90 %	0·50 %	77 : 1	,, 0·8 % ,,
4	,,	89 %	0·54 %	71 : 1	,, 1·8 % ,,
5	,,	88 %	0·57 %	67 : 1	,, 2·5 % ,,
6	,,	88 %	0·58 %	64 : 1	,, 3·1 % ,,
7	,,	87 %	0·62 %	60 : 1	,, 5·7 % ,,
8	,,	87 %	0·62 %	60 : 1	,, 6·8 % ,,
9	,,	84 %	0·81 %	45 : 1	,, 22·5 % ,,
10	,,	50 %	0·53 %	42 : 1	Red cells completely haemolysed at end of 7 hours.
11	Rabbit	42 %	0·96 %	17 : 1	Red cells completely haemolysed at end of 15 mins.

quinine bihydrochloride is 0·81 per cent., complete haemolysis was not attained after the lapse of three hours, while in Table 3, with a concentration of the quinine salt of 0·635 per cent. haemolysis was complete in three hours, the ratio of the weight of red blood cells to that of quinine bihydrochloride being, in the first case, about 45 to 1, and, in the second case, 36·4 to 1.

2. *Action of quinine in the form of alkaloid on red blood cells.*

So far the action upon red blood cells of quinine in the form of a salt has been investigated. Since quinine has not, however, been shown to be present in the blood in the form of a quinine salt, but may possibly be present in the free state, it was therefore decided to investigate the action of quinine in the alkaloidal form (Table 5). These experiments, owing to the sparing solubility of alkaloidal quinine, namely one in about sixteen hundred parts of water at 15° C., could only be carried out in very dilute solution. The series of experiments given in Table 5 is similar to those given in Table 1, except that the free alkaloid is employed instead of the quinine salt. Here it is seen that the amount of haemolysis is less than when the quinine salt was employed, the transition point, which as before is sharply marked, occurring with a concentration of alkaloid of 0·063 per cent., which is equivalent to 0·077 per cent. of quinine bihydrochloride, while the weight of red blood cells completely haemolysed is six and a half times that of the free quinine employed, or five and a quarter times that of the corresponding amount of quinine bihydrochloride.

TABLE 5. Haemolysis of red blood cells by quinine in alkaloidal state. Duration of experiment three hours. Temperature 37° C.

No. of Experiment	COMPOSITION OF MIXTURE OF RED BLOOD CELLS AND QUININE SOLUTION.					
	Quinine (alkaloid)	0·078 %	0·063 %	0·052 %	0·045 %	0·039 %
	$\frac{\text{Weight of wet red blood cells}}{\text{Weight of quinine (alkaloid)}}$	$\frac{4\cdot8}{1}$	$\frac{6\cdot4}{1}$	$\frac{8\cdot0}{1}$	$\frac{9\cdot6}{1}$	$\frac{11\cdot1}{1}$
1		Complete	Complete	Partial	Partial	Trace
2		Complete	Complete	Partial	Slight	Trace
3		Complete	Complete	Partial	Partial	Partial
4		Complete	Complete	Marked	Marked	Trace
5		Complete	Marked	Marked	Partial	Partial

It may here be pointed out that in these experiments quinine is present in colloidal solution, and that film formation on the liquid-air surface of the quinine solution is very marked. If such a solution is several times poured from one vessel to another a large amount of the quinine present may be separated out, and the concentration of the alkaloid correspondingly lowered. It is, therefore, necessary to avoid as far as possible any procedure likely to cause such separation of quinine. After the admixture of the solution of alkaloidal quinine with emulsion of red blood cells, the tendency to film formation is no longer observable.

3. *Action of hydrochloric acid on red blood cells.*

The quinine salt employed in the experiments recorded in Tables 1 to 3, when dissolved in water, becomes in part hydrolysed and forms an acid solution. It becomes therefore desirable to institute a comparison between the action of hydrochloric acid and of quinine upon red blood cells. In this connection, in order to admit of a wider range of comparison, the action of free alkali was also determined and may thus be contrasted with that of quinine in the form of free base. The latter experiments are given in the succeeding section.

TABLE 6. Haemolysis of red blood cells by hydrochloric acid dissolved in 0·9 per cent. NaCl solution. Duration of experiment three hours. Temperature 37° C.

No. of Experiment	COMPOSITION OF MIXTURE OF RED BLOOD CELLS AND HYDROCHLORIC ACID SOLUTION.					
	Hydrochloric acid	0·0137 %	0·0114 %	0·0098 %	0·0086 %	0·0076 %
	Weight of wet red cells	146	182	219	255	292
	Weight of hydrochloric acid	1	1	1	1	1
1		Complete	Complete	Partial	Slight	Slight
2		Complete	Complete	Partial	Slight	Slight
3		Complete	Complete	Marked	Slight	Slight
4		Complete	Complete	Complete	Slight	Slight
5		Complete	Complete	Marked	Slight	Slight

The experiments made with hydrochloric acid, which was dissolved in 0·9 per cent. sodium chloride solution, so as to be isotonic with blood plasma, are given in Table 6. These experiments form a continuation of those given in Table 2, with hydrochloric acid in place of quinine bihydrochloride. The action of hydrochloric acid in causing haemolysis is effective in much weaker concentration than is quinine bihydrochloride, the transition point being reached with 0·0114 per cent. of hydrochloric acid, while the corresponding weight of red blood cells haemolysed was one hundred and eighty-two times that of the hydrochloric acid present. The action of hydrochloric acid was not confined to the liberation of haemoglobin from the red blood cells. The liberated haemoglobin quickly underwent a further change, a soluble substance of brown colour, much more stable than haemoglobin, but giving no absorption bands on spectroscopic examination, making its appearance.

4. *Action of sodium hydrate on red blood cells.*

The action of caustic alkali on red blood cells differs from that of the preceding haemolytic agents, inasmuch as with it the transition point at which complete haemolysis ceases is not sharply defined, as will be seen on reference to Table 7, which, like Table 6, is a continuation of the series of experiments given in Table 2, but with sodium hydrate substituted for quinine bihydrochloride. In these experiments haemolysis was complete as far as a concentration of about 0·015 per cent. (chemically equivalent to 0·145 per cent. of quinine bihydrochloride). At this concentration the weight of red blood cells haemolysed was one hundred and sixty-six times the weight of sodium hydrate present. Thus, while the concentration of sodium hydrate in Table 7 is only one-tenth of that of quinine bihydrochloride in

TABLE 7. Haemolysis of red blood cells by sodium hydrate dissolved in 0·9 per cent. NaCl solution. Duration of experiment three hours. Temperature 37° C.

No. of Experiment	Composition of Mixture of Red Blood Cells and Sodium Hydrate Solution.				
	Sodium hydrate 0·021 %	0·018 %	0·016 %	0·015 %	0·014 %
	$\frac{\text{Weight of wet red cells}}{\text{Weight of sodium hydrate}}$ $\frac{113}{1}$	$\frac{133}{1}$	$\frac{153}{1}$	$\frac{166}{1}$	$\frac{173}{1}$
1	Complete	Almost complete	Marked	Marked	Marked
2	Complete	Almost complete	Marked	Marked	Marked
3	Complete	Complete	Complete	Complete	Marked
4	Complete	Complete	Complete	Complete	Marked
5	Complete	Complete	Complete	Complete	Marked

Table 2, yet the weight of red cells haemolysed by sodium hydrate is nearly ten times that haemolysed by the quinine salt during the same period of time at the same temperature.

5. *Action of quinine, hydrochloric acid and sodium hydrate on red blood cells compared.*

In the preceding observations the concentration of the various haemolytic agents has been given in percentage terms. For the sake of comparison all these results are grouped together in Table 8. The action of these agents, if it be of a chemical nature, will be a molecular action, and in this case the concentrations employed can be compared only if they are expressed in molar form. In Table 8, therefore, the molar concentration is given by the side of the percentage concentration, and similarly the maximum weight of red blood cells haemolysed is given not only in terms of the absolute weight of the haemolytic agent present, taken as a unit, but also in terms of a gramme-molecule of the latter, taken as a unit. In this Table, in addition to the results already given in the first seven Tables, an additional concentration of sodium hydrate at the transition point is also given, but it has not been thought necessary to give the complete table from which this result has been taken, for a further series of experiments, with slightly lower concentrations, is given in Table 11.

TABLE 8. Showing the relative amounts of human red cells completely haemolysed in three hours by quinine bihydrochloride, quinine in alkaloidal state, hydrochloric acid and sodium hydrate.

Table	Haemolytic agent	Concentration of haemolytic agent	Weight of wet red cells : Weight of haemolytic agent	Wet red cells (in grammes) : Haemolytic agent (gramme-molecule)
1	Q. 2 HCl	0·0450 % = 0·00113 M	10·4 : 1	4130 : 1
2	„	0·1550 % 0·00389 M	15·6 : 1	6200 : 1
3	„	0·6350 % = 0·01600 M	36·4 : 1	14430 : 1
5	Q. (alkaloid)	0·0628 % = 0·00198 M	6·4 : 1	2070 : 1
6	HCl	0·0114 % = 0·00320 M	182·0 : 1	6640 : 1
7	NaOH	0·0157 % = 0·00380 M	166·0 : 1	6640 : 1
	„	0·0090 % = 0·00226 M	133·0 : 1	5320 : 1

If the solutions employed to produce haemolysis are compared in respect of actual percentage of haemolytic agent present, it will be seen that hydrochloric acid is by far the most potent of the four made use of. Sodium hydrate comes close to hydrochloric acid, while quinine, even in higher concentrations, is a relatively feeble haemolytic agent, especially if employed in the alkaloidal state. When, however, the maximum weight of red blood cells completely haemolysed is compared, not with the actual weight of haemolytic agent present, but instead, with the weight of red blood cells haemolysed by a gramme-molecule of the haemolytic agent, then it is seen that, molecule for molecule, the above difference largely disappears, and the weight of wet red blood cells haemolysed varies in the different experiments within somewhat narrow limits. The results collected together in Table 8 are not, however, altogether suitable for close comparison since the molecular concentrations in the different series are not identical. In order to permit of a closer comparison, three additional series of experiments were carried out with quinine in the free state, hydrochloric acid and sodium hydrate, each series having the molar concentration 0·00113, and thus possessing the concentration of the transition point in Table 1. These experiments are given in Tables 9, 10 and 11, and the concentrations at the transition points are collected together in Table 12. No further reference is required at this point to Tables 9, 10 and 11. In Table 12 it is seen that, when equimolecular concentrations are employed, the weight of red blood cells completely haemolysed in three hours

TABLE 9. Haemolysis of wet red blood cells by quinine in alkaloidal state. Duration of experiment three hours. Temperature 37° C.

No. of Experiment	COMPOSITION OF MIXTURE OF RED BLOOD CELLS AND QUININE SOLUTION.					
	Quinine (alkaloid)	0·0366 %	0·0366 %	0·0366 %	0·0366 %	0·0366 %
	$\frac{\text{Weight of wet red cells}}{\text{Weight of quinine (alkaloid)}}$	$\frac{2{\cdot}4}{1}$	$\frac{3{\cdot}2}{1}$	$\frac{4{\cdot}8}{1}$	$\frac{6{\cdot}4}{1}$	$\frac{8{\cdot}0}{1}$
1		Complete	Complete	Marked	Partial	Slight
2		Complete	Complete	Complete	Marked	Marked
3		Complete	Complete	Complete	Very Marked	Partial
4		Complete	Complete	Complete	Marked	Slight
5		Complete	Complete	Complete	Almost complete	Marked

at 37° C. is in every case nearly the same, varying within very narrow limits, hydrochloric acid and sodium hydrate haemolysing in the concentration, given respectively 3,220 and 4,520 parts by weight of wet red blood cells per gramme-molecule of haemolytic agent, quinine in the alkaloidal form 1,550, and quinine bihydrochloride 4,130 parts of red cells. Quinine in the alkaloidal form, as already mentioned, is in colloidal solution in water. The low figure, which it gives, as compared with hydrochloric acid and sodium hydrate, which are in true solution and nearly completely ionised, may possibly be in part determined by the physical condition of hydrosol in which the alkaloidal quinine exists. On the other hand quinine bihydrochloride is in part hydrolysed, so that we have here to do with a triple effect, the solution being in reality a mixture of quinine salt, quinine in the

TABLE 10. Haemolysis of red blood cells by hydrochloric acid dissolved in 0·9 per cent. NaCl solution. Duration of experiment three hours. Temperature 37° C.

No. of Experiment	COMPOSITION OF MIXTURE OF RED BLOOD CELLS AND HYDROCHLORIC ACID					
	Hydrochloric acid	0·0041 %	0·0041 %	0·0041 %	0·0041 %	0·0041 %
	Weight of wet red cells	54·7	72·9	91·1	109	127
	Weight of hydrochloric acid	1	1	1	1	1
1		Complete	Complete	Complete	Slight	Trace
2		Complete	Complete	Complete	Slight	Trace
3		Complete	Complete	Complete	Slight	Trace

TABLE 11. Haemolysis of red blood cells by sodium hydrate dissolved in 0·9 per cent. NaCl solution. Duration of experiment three hours. Temperature 37° C.

No. of Experiment	COMPOSITION OF MIXTURE OF RED BLOOD CELLS AND SODIUM HYDRATE					
	Sodium hydrate	0·0045 %	0·0045 %	0·0045 %	0·0045 %	0·0045 %
	Weight of wet red cells	93	113	113	153	173
	Weight of sodium hydrate	1	1	1	1	1
1		Complete	Complete	Marked	Slight	Trace
2		Complete	Complete	Almost complete	Marked	Trace
3		Complete	Almost complete	Marked	Slight	Trace

alkaloidal form, and hydrochloric acid. On this account the production of a relatively greater degree of haemolysis is to be expected.* Taking all these facts into consideration, the results in Table 12 certainly suggest that in all cases the same process is at work, in other words, that whether haemolysis is caused by quinine salt, by quinine in the alkaloidal state, by hydrochloric acid, or by sodium hydrate, an identical reaction occurs. This point will be further referred to in the next section.

In addition to their haemolytic power the four substances in question may also be compared in respect of several other features. In the first place the sharpness of the transition point may be considered. This, as has already been mentioned, is well defined for quinine bihydrochloride, quinine in the free state and hydrochloric acid. If the series of tubes, in which the mixtures of haemolytic agent and red blood cells are contained, is examined at the end of three hours at 37° C., it is found that haemolysis is complete in all tubes up to the transition point. In the next tube there is more or less incomplete haemolysis. In the succeeding tube the haemolysis is slight, and in further tubes the supernatant liquid, left after centrifugalising, shows no indication of laking recognisable with the naked eye. With sodium hydrate, however, although the transition point can be recognised, there is no sudden cessation of haemolysis in the tubes lying beyond this point, but instead the degree of haemolysis lessens very gradually, most of the red cells present in the fifth or sixth tubes beyond that of the transition point being generally haemolysed.

TABLE 12. Comparison of the action on human red blood cells of quinine bihydrochloride, quinine in alkaloidal state, hydrochloric acid and sodium hydrate in the same molecular concentration. The amounts of red cells given were the largest amounts which could be completely haemolysed in three hours at 37° C.

Table	Haemolytic agent	Concentration of haemolytic agent	Weight of wet red cells : Weight of haemolytic agent	Wet red cells (in grammes) : haemolytic agent (gramme-molecule)
1	Q. 2 HCl	0·0450 % = 0·00113 M	10·4 : 1	4130 : 1
9	Q. (alkaloid)	0·0366 % = 0·00113 M	4·8 : 1	1550 : 1
10	HCl	0·0041 % = 0·00113 M	91·1 : 1	3220 : 1
11	NaOH	0·0045 % = 0·00113 M	113 : 1	4520 : 1

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* In 0·00113 M concentration quinine bihydrochloride is hydrolysed to the extent of about ten per cent.

A further difference between the haemolytic agents lies in their action in producing a further change in the haemoglobin liberated from the red cells. Hydrochloric acid produces the most marked effect, rapidly and, in the stronger solutions, in Table 6, completely changing the liberated haemoglobin, so that a brown solution results, which, as already mentioned, gives, on spectroscopic examination, no absorption bands. In less concentrated solutions the appearance of the brown coloration is less marked, but continues to increase when the tube is kept. Quinine bihydrochloride also produces the same change, especially in the more concentrated solutions which were employed, but in much less degree. In the more concentrated solutions of sodium hydrate also the brownish coloration is produced. On the other hand, quinine in the free state produces little change of this kind, the tubes in which it is contained not turning appreciably brown. Moreover, emulsions of red blood cells laked by free quinine exhibit little difference, as far as the haemoglobinometer reading is concerned, from emulsions of the same strength laked with distilled water, and the haemoglobin set free from red blood cells by alkaloidal quinine changes very slowly on keeping, so that the haemoglobinometer reading does not vary during the period of three hours to a degree sufficient to vitiate the experiment, as is the case with the remaining three haemolytic agents. Quinine bihydrochloride was found to cause the appearance of a relatively small amount of methaemoglobin.

A third point of considerable interest bearing on the nature of the process of haemolysis lies in the amount of haemolysis which the different concentrations of the haemolytic agents are capable of producing. Table 8 shows that the weight of red blood cells completely haemolysed in three hours by quinine bihydrochloride is approximately proportional to the square root of the concentration, for on reference to Experiments 1, 2 and 3 it is seen that the weight of red cells is 10·4, 15·6 and 36·4 that of the quinine salt, while the respective concentrations of the latter are 0·045 per cent., 0·155 per cent. and 0·635 per cent. If the weight of red blood cells completely haemolysed were exactly proportional to the square roots of the concentration of quinine salt, the first figures would be 10·4, 18·5 and 39·3 respectively, a difference which is well within the range of experimental error. For the concentrations of hydrochloric acid and sodium hydrate employed, which are well in the nighbourhood of the lowest concentration of quinine bihydrochloride, the weight of red blood cells completely haemolysed per unit weight of the haemolytic agent is also approximately proportional to the square root of the latter. Thus in the case of hydrochloric acid the maximum weight of wet red blood cells completely haemolysed in three hours in concentrations of 0·00114 per cent. and 0·0041 per cent. respectively is 182 and 91, the calculated amounts being 153 and 91. Similarly with concentrations of sodium hydrate of 0·0157 per cent., 0·0090 per cent. and 0·0045 per cent. the maximum weights of red blood cells completely haemolysed in three hours at 37° C. were respectively 166, 133 and 113, per unit weight of the haemolytic agent, the calculated amounts being 163, 136 and 113. In the case of quinine in the free state, although here, in consequence of the sparing solubility of the alkaloid and the limited range of concentration available, it has not been possible to determine this point with sufficient accuracy to enable an altogether satisfactory proof of the relationship between concentration and haemolytic power to be given, the concentrations employed being somewhat close together and the experimental error relatively large in consequence, yet the figures obtained—6·4 and 4·8 for the red cells and 0·0628 per cent. and 0·0366 per cent. for the concentrations indicate that the former is proportional to the square root of the latter, the calculated amounts for the red cells being 5·8 and 4·8 respectively.

6. *Nature of the action of quinine in the alkaloidal state on red blood cells.*

When haemoglobin is set free the action of the haemolytic agent is not directed to the red cell as a whole, for the cell is not chemically homogeneous, but to one of its constituents. Whether this constituent is haemoglobin itself or is part of the stroma, as in the case of haemolysins produced as antibodies by the injection of red cells into a foreign organism,* is not determined by our further experiments, which have instead been directed to the problem, what is the nature of the action of the haemolytic agents which have been used in the preceding investigation, in particular, is their action a chemical action, or does a purely physical process take place?

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* Ivar Bang und J. Forssman, Untersuchungen über die Hämolysinbildung. Zeitschr., f. d. ges. Biochemie, 1906. Bd. VIII, S. 238.

With a view to finding an answer to this enquiry, it was decided to determine the reaction rate at which haemolysis proceeds in the heterogeneous system formed by red blood cells suspended in liquid containing a haemolytic agent. This was found to be possible only in the case of quinine in the alkaloidal state, the remaining three haemolytic agents causing, in addition to haemolysis, decomposition of the liberated haemoglobin, and thus preventing the estimation of the amount liberated from being carried out.

The mode of procedure finally adopted for the determination of the reaction rate of quinine in the alkaloidal state was as follows:

Method. About 100 c.cm. of an emulsion of washed red cells in 0·9 per cent. sodium chloride solution containing 0·0358 per cent. = 0·00108 M. of quinine was prepared. This was placed in a water bath kept within ± 0·5° C. of the temperature selected for experiment. The emulsion was shaken up every fifteen minutes and at the end of an hour, and subsequently at half hourly intervals, samples of the emulsion measuring about 10 c.cm. were pipetted off and the percentage of haemoglobin (in terms of the equivalent amount of healthy red cells in the moist condition) present in the supernatant liquid after centrifugalisation, diluted if necessary with one to three parts of distilled water, determined by means of a haemoglobinometer, the absolute value of the readings of which had been previously ascertained in terms of the equivalent weight of red cells of one of us taken as a standard. Inasmuch as the laked haemoglobin undergoes a slight deterioration during the course of the experiment, a small correction, representing the mean of several determinations, and amounting to 0·5 division of the haemoglobinometer scale per hour, was applied to the readings. This correction is slight for the earlier haemoglobin estimations, and negligible for the later ones. The haemoglobinometer readings were readily made, the variations between successive readings of the same solution lying within ± 2 per cent. of the whole, and for readings lying between 25 and 65 on the scale being usually identical.

A series of estimations of the actual and percentage amounts of red blood cells haemolysed in successive periods of time is given in Table 13. The initial concentration of red cells in the emulsion was

0·476 per cent., and the percentage of quinine in the free state 0·0358 per cent. = 0·00108 M. To the results given in the second and third columns the formulae for bimolecular and multimolecular reaction rates were applied, but the values of K obtained were so divergent that these two reaction rates may be excluded from consideration. In Table 13 the reaction rate constant for monomolecular reactions, $K = \frac{1}{t_x - t_0} \log \frac{C_0}{C_x}$, is calculated in the last column The values obtained range between 0·0009 and 0·0047, the mean being 0·0022. The variations in the value of K are greatest at the beginning and end of the process of haemolysis, being low at the former and high at the latter. If, however, the period t_2 to t_7 is alone considered, the variations are much less, and during this period the reaction follows fairly closely a monomolecular rate.

TABLE 13. The rate of haemolysis of red blood cells by a 0·0358 % solution of quinine in 0·9 % solution of sodium chloride. The ratio of the weight of wet red cells to the amount of quinine present is 12·6 : 1. Temperature of experiment 37° C.

Time t_x		Concentration of wet red cells	Same in percentage of 0·476		$\frac{1}{t_x - t_0} \log \frac{C_0}{C_x}$
t_0	0 min.	0·476 %	100·0		
t_1	50 ,,	0·432 %	90·8		0·0009
t_2	80 ,,	0·396 %	82·0		0·0011
t_3	110 ,,	0·341 %	71·7		0·0013
t_4	140 ,,	0·258 %	54·2		0·0019
t_5	170 ,,	0·188 %	39·5		0·0024
t_6	200 ,,	0·146 %	30·7		0·0021
t_7	230 ,,	0·074 %	21·2		0·0029
t_8	260 ,,	0·029 %	6·1		0·0047
				Mean	0·0022

TABLE 14. Reaction rate constant of the experiments recorded in Table 13 recalculated.

Time		Concentration of wet red cells	Same in percentage of 0·476	$\frac{1}{t_{n+1} - t_n} \log \frac{C_n}{C_{n+1}}$	
t_0	0 min.	0·476 %	100·0		
t_1	50 ,,	0·432 %	90·8	0·0009	
t_2	80 ,,	0·396 %	82·0	0·0015	
t_3	110 ,,	0·341 %	71·7	0·0019	
t_4	140 ,,	0·258 %	54·2	0·0040	Mean
t_5	170 ,,	0·188 %	39·5	0·0045	t_2 to t_7
t_6	200 ,,	0·146 %	30·7	0·0037	0·0039
t_7	230 ,,	0·074 %	21·2	0·0054	
t_8	260 ,,	0·029 %	6·1	0·0183	

The mode of calculating K adopted in Table 13, namely by determining K for a series of periods, each of which commences at the same point of time, that is to say, the time at which the experiment is begun, is well adapted for showing progressive variations in the values of K, since in this way the irregularities due to error of estimation become less accentuated. The latter are greatest when K is calculated for every pair of successive estimations, as is done in Table 14, where the values obtained present greater irregularities than in Table 13. The method of calculation adopted in Table 14 gives a more correct average for the period t_2 to t_7, than is obtainable by the method employed in Table 13. In Table 15 the percentages of red cells haemolysed are calculated from the average value of K between t_2 and t_7 obtained in Table 14. It is seen that within this period there is a close correspondence between the calculated and observed percentages. A comparison of the calculated and observed results,

TABLE 15. Comparison of percentages found in Table 13 and those calculated from the value of K = 0·0039.

Time	$t_n + 1 - t_n$	K $(t_n + 1 - t_n)$	Concentration of wet red cells	
			Calculated	Found
t_0 0 min.			[187·2]	[100]
t_1 50 ,,	50 min.	0·0039 × 50 = 0·195 = log 1·57 = log $\frac{187\cdot2}{119\cdot2}$	[119·2]	[90·8]
t_2 80 ,,	30 ,,	,, 30 = 0·117 = log 1·31 = log $\frac{119\cdot2}{91\cdot2}$	[91·2]	[82·0]
t_3 110 ,,	30 ,,	,, ,, ,, = log $\frac{91\cdot2}{69\cdot5}$	69·5	71·7
t_4 140 ,,	30 ,,	,, ,, ,, = log $\frac{69\cdot5}{53\cdot1}$	53·1	54·2
t_5 170 ,,	30 ,,	,, ,, ,, = log $\frac{53\cdot1}{40\cdot5}$	40·5	39·5
t_6 200 ,,	30 ,,	,, ,, ,, = log $\frac{40\cdot5}{30\cdot9}$	30·9	30·7
t_7 230 ,,	30 ,,	,, ,, ,, = log $\frac{30\cdot9}{23\cdot6}$	23·6	21·1
t_8 260 ,,	30 ,,	,, ,, ,, = log $\frac{23\cdot6}{18\cdot1}$	[18·1]	[6·1]

preceding and following this period, which are given in brackets, shows, as is also seen in Tables 13 and 14, the extent to which at the beginning haemolysis proceeds slowly and towards the end progresses with increased rapidity. The cause of this initial delay and final acceleration of the process of haemolysis is not clear; it may perhaps be dependent upon the physical conditions under which the interaction of cell and haemolytic agent occurs. It is, however, established by the above experiments that the reaction in question during the greater part of its course resembles a chemical reaction monomolecular in character, presenting, however, an initial retardation and final acceleration.

In Table 16 the series of experiments given in Table 13 is repeated, and seven other series added. In these the reaction rate constant is calculated for the period t_1 to t_8, the starting point being t_1 instead of t_0 as in Table 13. This method of calculation has been adopted because of the difficulty of ascertaining the precise moment at which the temperature of the emulsion becomes identical with that of the water in the bath. In these different series of experiments haemolysis is seen to follow pretty closely a monomolecular rate except at the beginning and end of the reaction, when the respective slowing and acceleration already referred to occur, while on the other hand a bimolecular or multimolecular rate is excluded by the much wider range of variation in the value of the constants calculated for such reaction rates. The actual reaction rate as shown by the values of K varies from day to day even in the same healthy individual, the variations being greater in the series Y_1 to Y_4 than in the series B_1 to B_4. The cause of these variations, which appear to lie outside the range of experimental error, is obscure. It is true that variations occur in the red blood cells employed in different experiments. In particular, it was constantly found that although the percentage weight of wet red cells in the emulsions was always the same, namely, 0·444 per cent., being determined by means of the haemocrit, yet when the red blood cell content of the emulsions was determined (after laking with distilled water) by means of a haemoglobinometer reading the result was different in each case Thus in the experiments recorded in Table 16 the haemoglobinometer determinations of the emulsions were found to vary from 0·476 per cent. to 0·532 per cent. These variations are too large to be accounted for as due to

TABLE 16. The rate of haemolysis of red blood cells by quinine in the alkaloidal state. Conditions of experiment as in Table 13.

Time	Y 1			Y 2			Y 3			Y 4		
	Concentration of wet red cells.	Same in percentage form	$\frac{1}{t_x - t_1} \log \frac{C_1}{C_x}$	Concentration of wet red cells.	Same in percentage form	$\frac{1}{t_x - t_1} \log \frac{C_1}{C_x}$	Concentration of wet red cells.	Same in percentage form	$\frac{1}{t_x - t_1} \log \frac{C_1}{C_x}$	Concentration of wet red cells.	Same in percentage form	$\frac{1}{t_x - t_1} \log \frac{C_1}{C_x}$
t_0 0 min.	0·496 %	100·0	—	0·492 %	100·0	—	0·524 %	100·0	—	0·532 %	100·0	—
t_1 50 ,,	0·435 %	87·7	—	0·391 %	79·5	—	0·442 %	84·2		0·417 %	85·9	—
t_2 80 ,,	0·368 %	74·2	0·0024	0·275 %	55·9	0·0051	0·353 %	67·3	0·0017	0·415 %	78·0	0·0014
t_3 110 ,,	0·286 %	57·7	0·0030	0·146 %	29·7	0·0071	0·252 %	48·1	0·0040	0·370 %	69·5	0·0015
t_4 140 ,,	0·215 %	43·3	0·0034	0·087 %	19·5	0·0068	0·149 %	28·4	0·0052	0·299 %	56·2	0·0020
t_5 170 ,,	0·142 %	38·7	0·0028	0·048 %	9·7	0·0084	0·098 %	18·7	0·0054	0·266 %	42·4	0·0025
t_6 200 ,,	0·109 %	22·0	0·0032	0·021 %	4·3	0·0084	0·062 %	11·8	0·0057	0·136 %	25·5	0·0034
t_7 230 ,,	0·058 %	11·7	0·0049	0·018 %	3·6	0·0084	0·038 %	7·2	0·0060	0·073 %	13·3	0·0045
t_8 260 ,,	0·018 %	3·6	0·0065	0·015 %	3·0	0·0068	0·014 %	2·7	0·0071	0·040 %	7·5	0·0029
t_9 290 ,,	—	—	mean	0·003 %	0·6	0·0088	0·008 %	1·5	0·0073	—	—	—
			0·0037			mean			mean			mean
						0·0071			0·0053			0·0026
	B 1			B 2			B 3			B 4		
t_0 0 min.	0·476 %	100·0	—	0·504 %	100·0	—	0·496 %	100·0	—	0·500 %	100·0	—
t_1 50 ,,	0·432 %	90·8	—	0·455 %	90·2	—	0·456 %	91·8	—	0·434 %	86·8	—
t_2 80 ,,	0·396 %	82·0	0·0015	0·410 %	81·2	0·0015	0·425 %	85·7	0·0010	0·407 %	81·4	0·0010
t_3 110 ,,	0·341 %	71·7	0·0019	0·319 %	63·2	0·0026	0·372 %	75·0	0·0014	0·381 %	76·2	0·0009
t_4 140 ,,	0·258 %	54·2	0·0025	0·252 %	50·0	0·0028	0·281 %	56·7	0·0023	0·315 %	63·0	0·0016
t_5 170 ,,	0·188 %	39·5	0·0030	0·177 %	35·1	0·0034	0·196 %	39·5	0·0030	0·260 %	52·0	0·0018
t_6 200 ,,	0·146 %	30·7	0·0031	0·084 %	16·7	0·0050	0·127 %	25·6	0·0037	0·203 %	40·6	0·0023
t_7 230 ,,	0·074 %	21·2	0·0035	0·060 %	11·9	0·0049	0·049 %	9·9	0·0042	0·134 %	26·8	0·0029
t_8 260 ,,	0·029 %	6·1	0·0056	0·033 %	6·5	0·0054	0·013 %	2·6	0·0073	0·056 %	11·0	0·0043
			mean			mean			mean			mean
			0·0030			0·0036			0·0033			0·0021

error of experiment. Moreover, such diurnal variations in health have their counterpart in the much larger variations in disease, as will be seen later in Table 24. Here we have evidence of variation in the content of haemoglobin per unit volume of wet red blood cells which is capable of independent variation in health. When, however, the variations in haemoglobin in different samples of blood from the same individual in Table 16 are compared with corresponding values of K, it is seen that no constant relationship between the two is recognisable. The mean value of K at 37° C. for the Y series is 0·0035, and for the B series 0·0030.

In monomolecular reaction rates at different temperatures the relation

$$\frac{K_2}{K_1} = e^{\frac{\mu}{2}\left(\frac{1}{\tau_1} - \frac{1}{\tau_2}\right)}$$

holds, τ_1 and τ_2 being the absolute temperatures at which two estimations of K are carried out. To ascertain if this relation held for the haemolysis of red cells by quinine, the series of experiments recorded in Table 17 were carried out at temperatures of 37° C., 31° C. and 25° C. respectively, the concentration of quinine (in the alkaloidal state) being 0·00113 M. (0·0366 per cent.). In order to avoid the effect of the diurnal variations in the composition of the red cells, illustrated in Table 16, each of the three sets of determinations (Experiments 1 to 4, Table 17) was carried at the same time with portions of the same emulsion of red cells in quinine solution. In Table 18, with the aid of the values of K obtained from Table 17, the value of μ was determined. Before proceeding to consider the value of the latter constant, however, a few remarks may be made upon the data obtained in the former Table.

In the first place, it is to be observed that the same initial delay and terminal acceleration of the process of haemolysis, which has already been noted at 37° C. in Table 16, occurs also at 31° C. and 25° C. When the concentration of the red cells has been reduced by haemolysis to about 80 per cent., the reaction proceeds at all three temperatures at a tolerably constant rate until the concentration of red cells reaches about 20 per cent. Between these limits the values of K, calculated for a monomolecular rate, do not exhibit any very great variation; when calculated for a bimolecular or multimolecular reaction rate, the variations obtained are sufficiently great to exclude

TABLE 17. Rate of haemolysis of red blood cells, at different temperatures, by a ·00113 M (·0366 %) solution of quinine in the alkaloidal state.

No. of Experiment	t	Per-centage of haemoglobin in red cells	T 37° C. $\frac{1}{t_{n+1}-t_n}\log\frac{C_{n+1}}{C_n}$	Percentage of haemoglobin in red cells calculated from value of K	t	Per centage of haemoglobin in red cells	T 31° C. $\frac{1}{t_{n+1}-t_n}\log\frac{C_{n+1}}{C_n}$	Percentage of haemoglobin in red cells calculated from value of K	t	Per-centage of haemoglobin in red cells	T 25° C. $\frac{1}{t_{n+1}-t_n}\log\frac{C_{n+1}}{C_n}$	Percentage of haemoglobin in red cells calculated from value of K
1	0 min.	100		(175) Value of	0 min.	100*		(229)	0 min.	100		(189)
	90 ,,	41	0·0043 Mean	37 K taken at	90 ,,	84·6	0·0008*	(88)	150 ,,	75·8	0·0021	72 Value of
	135 ,,	12·3	0·0116 0·0078	19 0·0074	135 ,,	55·7	0·0040 Mean	55 Value of	210 ,,	48·8	0·0032 Mean	49 K taken at
	180 ,,	2*	0·0156*	(8) (Table 18)	180 ,,	35·1	0·0045 0·0043	33 K taken at	270 ,,	32 9	0·0028 0·0032	33 0·0028
					210 ,,	18·7*	0·0091*	25 0·0046	300 ,,	22·2	0 0057	27·5 (Table 18)
					255 ,,	6·9*	0·0144*	(15·5) (Table 18)				
2	0 ,,	100		(119)	0 ,,	100		(115)				
	60 ,,	45·5	0·0057 Mean	45 Value of	90 ,,	46·5	0·0037 Mean	46 Value of				Value of K
	90 ,,	25	0·0087 0·0071	28 K taken at	120 ,,	32·5	0·0051 0·0044	34 K taken at				0·0027
	120 ,,	7*	0·0184*	(17) 0·0071	150 ,,	10*	0·0170*	25 0·0044				(Table 18
	150 ,,	4*	0·0081*	(10) (Table 18)	180 ,,	2*	0·0223*	(18·5) (Table 18)				
3	0 ,,	100*		(280)	0 ,,	100*		(220)	0 ,,	100*		(154)
	30 ,,	93·3*	0·0011*	(153) Value of	90 ,,	71·6	0·0016	72	80 ,,	90·7*	0·0005	(83)
	60 ,,	89·3	0·0007*	84 K taken at	120 ,,	52·2	0·0046	49 Value of	105 ,,	79·8	0·0022	70 Value of
	90 ,,	43·8	0·0102 0·0102	46 0·0087	150 ,,	34·4	0·0061 Mean	34 K taken at	135 ,,	58	0·0046	55 K taken at
	120 ,,	18·8*	0·0123*	25·5 (Table 18)	165 ,,	26·6	0·0044 0·0047	28 0·0054	165 ,,	45·9	0·0033 Mean	44 0·0033
	150 ,,	6·5*	0·0153	(14)	195 ,,	11·8*	0·0177	(19·5) (Table 18)	195 ,,	34·8	0·0040 0·0040	35 (Table 18)
					225 ,,	8·0*	0·0057	(13·5)	225 ,,	25·9	0·0042	28
					235 ,,	4·5*	0·0083	(9)	255 ,,	11*	0·0057	(22)
4	0 ,,	100*		(222)	0 ,,	100*		(292)	0 ,,	100*		(229)
	105 ,,	92·6*	0·00031	(112)	165 ,,	91·7*	0·00022	(157)	270 ,,	90·3*	0·00016	(126)
	135 ,,	87·9*	0·00070	(93·5)	195 ,,	90·3*	0·00027	(140)	330 ,,	88·6*	0·00013	(107)
	165 ,,	83·4*	0·00070	78	225 ,,	88·3*	0·00027	(125)	360 ,,	85·8*	0·00013	(99·5) Value of
	195 ,,	75·2	0·00150	65	255 ,,	85·6*	0·00040	(112) Value of	390 ,,	84·1*	0·00013	(93) K taken at
	225 ,,	62·9	0·00257	54 Value of	285 ,,	82·8*	0·00040	(105) K taken at	480 ,,	74·8	0·00065	76 0·0010
	255 ,,	50·0	0·00333 Mean	45 K taken at	315 ,,	78·6	0·00070	(90) 0·0016	510 ,,	69·5	0·00110	70·5 (Table 18
	285 ,,	37·8	0·00400 0·0030	38 0·0026	345 ,,	72·2	0·00123	(81) (Table 18)	540 ,,	66·1	0·00070 Mean	66
	315 ,,	31·2	0·00273	32 (Table 18)	375 ,,	68·9	0·00070	72·5	570 ,,	63·2	0·00070 0·00095	62
	345 ,,	26·5	0·00240	27	405 ,,	62·5	0·00137	64·5	600 ,,	59·5	0·00150	57·5
	375 ,,	18·3*	0·00267	22	450 ,,	56·8	0·00091 Mean	55	660 ,,	53·4	0·00075	50
	435 ,,	13·7*	0·00433	(15)	480 ,,	46·6	0·00290 0·0015	49				
					510 ,,	43·8	0·00150	44				
					540 ,,	36·4	0·00263	39				
					570 ,,	32·4	0·00197	35				
					600 ,,	31·8	0·00027	32				
					660	26·8	[illegible]	28				

these reaction rates. Above the upper limit the values of K for a monomolecular reaction rate become increasingly small, and below the lower limit they become increasingly high. The marked character of the initial retardation and final acceleration is also well illustrated when the percentages (enclosed in brackets in Table 17), lying without the limits just given, are calculated according to the mean values of K obtaining within these limits. Two interpretations may be placed upon the different course taken by the reaction at its commencement and its termination: it may be that the reaction rate is, in itself, complex in character, becoming simple and monomolecular in one stage of its course; or it may be that the physical conditions, under which the reaction takes place, are such as to influence its rate at the beginning and again towards the end of the haemolysis, but not to any considerable extent in the rest of its course. In favour of the latter interpretation is the fact that, on the one hand alkaloidal quinine is in colloidal solution, and, therefore, cannot enter into a chemical reaction so readily as if it were in true solution, while on the other hand the red cell is surrounded externally by a membrane, which may offer physical difficulties to the entrance of the haemolytic agent into its interior.

TABLE 18. Determination of value of μ from experiments recorded in Table 17.

No. of Experiment.	Temperature of experiment	Value of K determined by experiment (Table 17)	Value of μ calculated from formula $\frac{K_2}{K_1} = e^{\frac{\mu}{2}\left(\frac{T_2 - T_1}{T_2 T_1}\right)}$			Percentage of red cells at beginning of experiment determined by haemoglobinometer	Value of K calculated from $\mu = 15{,}000$
1	37° C.	0·0078	$t_{37°}$ and $t_{31°}$	14500	mean 11600	0·408	0·0074
,,	31° C.	0·0043	$t_{37°}$ and $t_{25°}$	11500		0·408	0·0046
,,	25° C.	0·0032	$t_{31°}$ and $t_{25°}$	8800		0·408	0·0028
2	37° C.	0·0071	$t_{37°}$ and $t_{31°}$	15000		0·400	0·0071
,,	31° C.	0·0044				0·400	0·0044
,,	25° C.					0·400	0·0027
3	37° C.	0·0102	$t_{37°}$ and $t_{31°}$	23000	mean 14000	0·540	0·0087
,,	31° C.	0·0047	$t_{37°}$ and $t_{25°}$	14000		0·540	0·0054
,,	25° C.	0·0040	$t_{31°}$ and $t_{25°}$	4000		0·540	0·0033
4	37° C.	0·0030	$t_{37°}$ and $t_{31°}$	22000	mean 18000	0·528	0·0026
,,	31° C.	0·0015	$t_{37°}$ and $t_{25°}$	18000		0·528	0·0016
,,	25° C.	0·00095	$t_{31°}$ and $t_{25°}$	14000		0·528	0·0010

Presumably dependent on the same cause are the variations in the values of K observable in the individual experiments in Table 17. These are greater than those in Table 16, ranging at 37° C. between 0·0030 and 0·0102 (or, when recalculated from the mean value of μ, between 0·0026 and 0·0087). Table 17 shows that, in each emulsion these individual variations characterise the reaction rate at all temperatures employed; thus if the rate is rapid at 37° C., it is also rapid at 31° C. and at 25° C., and if relatively slow at the first temperature it is also relatively slow at the lower temperatures. This relationship is seen at a glance in Table 19, which illustrates the necessity of making simultaneous observations of the reaction rate of the same emulsion at different temperatures in order to determine the value of μ; obviously values of K taken at random from different experiments are of no value for this purpose. It will be noticed also that, owing to the large number of observations which require to be made, and the rapidity with which reaction proceeds at 37° C., some difficulty is experienced in obtaining a sufficient number of estimations lying between 80 per cent. and 20 per cent. at this temperature. The values lying beyond these limits, which are neglected in calculating the mean value of K, are indicated, in the Table, with an asterisk. In Table 18 the initial percentages of red cells in the different experiments are given; no definite relation between the concentration of red cells employed and the values of K or μ is to be observed.

TABLE 19. Comparison of values of K at different temperatures.

T	No. of Experiment.				Calculated for $\mu = 15000$				Mean
	1	2	3	4	1	2	3	4	
37° C.	0·0078	0·0071	0·0102	0·0030	0·0074	0·0071	0·0087	0·0026	0·0065
31° C.	0·0043	0·0044	0·0049	0·0015	0·0046	0·0044	0·0054	0·0016	0·0040
25° C.	0·0032	—	0·0040	0·00095	0·0028	0·0027	0·0033	0·0010	0·0024

In Table 18 the value of μ is determined, separately for each experiment, from the data given in Table 17, by the aid of the formula given on p. 33. In each experiment three values of μ are obtained, the mean of which is taken as the true value. Although the mean values show some variation, being highest in Experiment 4, in which haemolysis proceeded relatively slowly, and low in Experiments 1 and 3, in which haemolysis was relatively rapid, yet these variations are inconsiderable, the values of μ lying within a narrow range, the highest mean value being 18,000 and the lowest 11,600, the mean of all being 15,000. From the latter value of μ the lowering of the temperature required to diminish the rate of reaction to one-half may be calculated; at 37° C. it amounts to 9° C. With the aid of this mean value of 15,000 the percentages in Experiments 1 to 4, Table 17, are re-calculated, the values of K thus obtained not differing greatly from those determined by experiment. The difficulties in the way of carrying out these experiments, it may be observed, are by no means inconsiderable.

Additional confirmation of the conclusion already reached (p. 31), that haemolysis produced by the action of quinine in the alkaloidal form resembles a chemical process, not a physical one, is thus afforded by the circumstance that approximately identical values of μ are obtainable in the experiments at varying temperatures recorded in Table 18.

The fact that, in the heterogeneous system formed by red blood cells suspended in a hydrosol of alkaloidal quinine, haemolysis progresses, except at the beginning and end of the process, at a monomolecular rate, indicates that quinine behaves like a catalytic agent, its action being similar to a ferment action comparable, for example, to that of pepsin. Quinine bihydrochloride, hydrochloric acid and sodium hydrate presumably also act like catalytic agents. The resemblance of these haemolytic agents to pepsin is all the greater when it is borne in mind that the amount of red cells completely haemolysed in three hours at 37° C. is proportional to the square root of the concentrations employed, for precisely the same relationship holds, within certain limits, for pepsin.* The value of μ for the digestion of gelatine by pepsin was found by Sjöqvist to be 10,750, and for the digestion of egg-albumin by pepsin to be 15,570. These values are not far removed from those obtained for the haemolysis of red cells by quinine.

It is to be anticipated that, as quinine in the free state, in producing haemolysis of red blood cells, resembles a catalytic agent, it is not used up to any considerable extent in the process, and that its concentration is therefore little altered by the reaction taking place. To test this point the series of estimations recorded in Tables 20 and 21 were made. In these estimations a weighed quantity of quinine sulphate was dissolved in such an amount of 0·9 per cent. sodium chloride solution as to produce about the same concentration as that employed in the experiments given in Table 1. Red cells were then added. In the first four experiments the weight of red blood cells taken was comparable to that in Table 1, in the remaining experiments much larger amounts of red cells were employed. When it was judged that sufficient time had elapsed for the red cells to take up quinine, the amount of the latter still remaining in solution was determined.

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* Sjöqvist, Scandin. Archiv. f. Physiologie, 5 (1895).

Method. In these estimations healthy human blood was employed throughout, being withdrawn by means of a sterilised glass injection syringe, from the cephalic, median-cephalic or ulnar vein of the upper limb, which was bandaged close to the axilla so as to obstruct the venous circulation. The time occupied in withdrawing blood was from ten to thirty seconds, and the amount withdrawn ranged from 4 c.cm. to 27 c.cm. Without any delay the blood so obtained was discharged into a beaker containing a freshly prepared 1 per cent. solution of potassium oxalate, in the proportion of five parts of the former to one part of the latter, and the mixture at once centrifugalised. The red cells quickly subsided and the supernatant serum was pipetted off. The red cells were then added in measured amounts to a solution of quinine sulphate, the bulk of which was about 200 c.cm. The emulsion was kept in a stoppered glass bottle which was shaken every half hour for two hours, after which the red cells were allowed to subside at room temperature (17° C. to 19° C.), subsidence being completed in sixteen to twenty-four hours. No haemolysis took place. As much as possible of the supernatant liquid, which was clear or, with the larger amounts of red cells, very faintly opalescent, was removed with the aid of a capillary siphon, and the amount of fluid still remaining, usually not exceeding 5 per cent. of the original amount, was measured. It will be noted that the quinine solution after the first two hours was not everywhere equally exposed to the further action of the red cells, these gradually disappearing from the upper part of the liquid and becoming increasingly dense below.

The amount of quinine in the supernatant liquid so obtained was then estimated, and from this the total amount of quinine still remaining in solution determined. The method of estimation employed was that of Giemsa and Schaumann,* slightly modified to suit the conditions under which our investigation was carried out.

To the supernatant liquid, which was contained in a stoppered glass bottle holding 300 c.cm., about 2 g. of solid potassium hydrate were added. When the potassium hydrate had dissolved, the liquid had in most cases become milky in aspect, though no precipitate was recognisable. 70 c.cm. of ether were now added, the milkiness at once disappearing. The mixture of ether and watery solution was then shaken for half an hour. If the ether did not separate out, a small amount of alcohol was added, when ready separation took place. The ether was then transferred to a flask, by means of a capillary siphon, the amount of the upper layer of ether left behind being one-tenth to one-twentieth. Ether was now added again in three successive amounts of 50 c.cm., and siphoned off as before, in each case after half an hour's shaking. It may here be mentioned that three extractions with ether as recommended by Giemsa and Schaumann were not always sufficient to remove the last traces of quinine. The ether was then distilled off and the residue dried in a water oven at 98° C. When quite dry the residue was taken up with chloroform. The chloroform extract was put up in a weighing bottle, allowed to evaporate in a water oven at 70° C., then kept at 120° C. for one hour and weighed. The residue thus obtained was glassy in appearance, and colourless or of a faint brownish tint. It was found necessary to re-distil the ether and chloroform, and to test for the presence of any non-volatile residue before use. In the hands of Giemsa and Schaumann† three estimations of 0·05 g., 0·1 g. and 0·02 g. of quinine respectively gave an experimental error of − 0·4 per cent., + 1·0 per cent. and − 1·2 per cent. In our estimations two control amounts of 0·1100 g. are recorded. with an experimental error of – 2·6 per cent. and – 1·5 per cent. respectively.

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* Pharmakalogische und chemisch-physikalische Studien über Chinin. Arch. f. Schiffs- u. Tropenhygiene, 1907, Bd. XI, S. 7.

† Loc. cit. p. 18.

The observations recorded in Tables 20 and 21 show that under the conditions of experiment obtaining, which, as already mentioned, were similar to those in Table 1, namely with a quinine sulphate solution of about 0·05 per cent. concentration and with an amount of red blood cells in such excess as to prevent any trace of haemolysis during the course of the experiment, quinine is withdrawn from solution by red cells. In the first four experiments the withdrawal of quinine from solution by the action of the red cells is not satisfactorily exhibited since the amount taken up lies too near the error of experiment. When, however, the weight of red cells, instead of being 1·5 g. to 2·6 g. [Experiments 1 to 4, Table 20] is from 7·9 g. to 12 g., then the amount of quinine withdrawn, though relatively small, lies altogether beyond the range of experimental error and becomes readily ascertainable, varying between 11 per cent. and 22 per cent. of the amount originally present (Table 23). It is thus clear that the red cells take up quinine, and the amount which is combined is found (Experiments 5, 6 and 7) to be about 0·14 per cent. If we assume that the amount of quinine taken up by varying amounts of red cells, when the final concentration of quinine in the

TABLE 20. Determination of the amount of quinine withdrawn from solution by healthy human red blood cells. Duration of action of red blood cells on quinine sulphate dissolved in 0·9 per cent. solution of sodium chloride eighteen to twenty-four hours. Temperature of experiment, 17° C. to 19° C.

No. of Experiment	Amount of quinine sulphate taken	Equivalent amount of quinine	Concentration of quinine sulphate	Amount of quinine recovered	Percentage of quinine recovered	Weight of wet red cells taken	Weight of wet red cells : Weight of quinine sulphate
1	0·1263 g.	0·1098 g.	0·0473 g.	0·1050 g.	95·6 %	1·5 g.	12 : 1
2	0·1263 g.	0·1098 g.	0·0547 g.	0·1047 g.	95·4 %	1·8 g.	14 : 1
3	0·1263 g.	0·1098 g.	0·0522 g.	0·1071 g.	97·5 %	2·2 g.	17 : 1
4	0·1193 g.	0·1037 g.	0·0529 g.	0·1023 g.	98·8 %	2·6 g.	22 : 1
5	0·1263 g.	0·1098 g.	0·0541 g.	0·0985 g.	88·7 %	7·9 g.	63 : 1
6	0·1193 g.	0·1037 g.	0·0534 g.	0·0907 g.	87·7 %	10·7 g.	90 : 1
7	0·1189 g.	0·1032 g.	0·0571 g.	0·0808 g.	78·3 %	12·0 g.	101 : 1
8 control	0·1267 g.	0·1100 g.	0·0517 g.	0·1071 g.	97·4 %	—	—
9 control	0·1267 g.	0·1100 g.	0·0517 g.	0·1084 g.	98·5 %	—	—

surrounding fluid is in all cases the same, is proportional to the weight of red cells present, then in a final concentration of 0·0404 per cent. the amount of quinine taken up by 2 g. of wet red cells would be about 3 per cent. of the amount originally present in solution, under the conditions of experiment obtaining in Table 20. In view of the results obtained in the comparative experiments recorded in Table 9, it may be doubted if this simple relation holds. It may be suggested that the quantity taken up by the smaller amounts of red cells would probably be relatively greater than that taken up by the larger amounts in Tables 20 and 21. Unfortunately the determination of the amount taken up by small quantities of red cells, representing the small difference of two estimations, each subject to an error of experiment, which is not of negligible dimensions, cannot be carried out with sufficient accuracy to settle this point.

TABLE 21. Determination of the percentage amount of quinine, contained in red blood cells in the experiments recorded in Table 20.

No. of Experiment	Concentration of quinine sulphate originally present	Weight of wet red cells : Weight of quinine present at beginning of experiment	Percentage of the quinine, originally present, which was withdrawn from solution by red cells	Percentage of quinine in wet red cells	Final concentration of quinine in liquid part of emulsion of red cells
1	0·0473 % = 0·0412 % quinine	13·7 : 1	(4·4 %)	(0·320 %)	0·0393 %
2	0·0547 % = 0·0475 % ”	16·4 : 1	(4·6 %)	(0·284 %)	0·0453 %
3	0·0522 % = 0·0453 % ”	20·0 : 1	(2·5 %)	(0·123 %)	0·0442 %
4	0·0529 % = 0·0460 % ”	25·0 : 1	(1·2 %)	(0·054 %)	0·0455 %
5	0·0541 % = 0·0470 % ”	89·7 : 1	11·3 %	0·143 %	0·0417 %
6	0·0534 % = 0·0464 % ”	103·3 : 1	12·3 %	0·121 %	0·0407 %
7	0·0571 % = 0·0496 % ”	116·3 : 1	21·7 %	0·187 %	0·0388 %
mean of	5, 6 and 7 = 0·0477 quinine			mean of 5, 6 and 7 = 0·137 %	mean of 5, 6 and 7 = 0·0404 %

Returning now to the enquiry, which formed the starting point of the experiments in Tables 20 and 21, namely, whether quinine is used up to any considerable extent when acting upon red cells so as to cause haemolysis, we find that under the conditions obtaining in the experiments recorded in Table 1 the concentration of the quinine solution undergoes a diminution, but that the extent of the diminution is very small. The conclusion arrived at on p. 38 is therefore confirmed.

A further relation exists to which reference may be made. So long as the haemolysis of red cells proceeds at a monomolecular rate the amount of red cells haemolysed during any short period of time is closely proportional to the concentration of the red cells in the emulsion at the beginning of the period. This is seen to be the case for Table 15, in which the weight of red cells haemolysed each half hour is found to be, for the calculated amounts, almost exactly 0·236 of the concentration at the beginning of each period [thus $91{\cdot}2 \quad 69{\cdot}5 = 21{\cdot}7 - 91{\cdot}2 \times 0{\cdot}238$, and so on].

7. *Action of quinine on healthy red cells in the living body.*

The experiments recorded in Table 4 show that quinine bihydrochloride scarcely has any action upon red blood cells until its concentration reaches 0·5 per cent. If the percentage of quinine salt falls below this amount extremely little change takes place on keeping three hours at 37° C. Assuming the amount of blood in the human body to be 5,000 c.cm., the amount of quinine bihydrochloride required to produce a concentration of 0·5 per cent. would be 25 grammes (387 grains), that is 0·3 g. per kilo of body weight, if the quinine were introduced intravenously. Since a dose of this amount, which would be rapidly fatal, is far above the maximum amount taken

TABLE 22. Quinine in relation to blackwater fever.

Case	Amount of quinine taken	Relation to appearance of blackwater
A	8 grs. at mid-day, 8 grs. at 3 p.m.	Passed blackwater at 6 p.m.
B	Two 10-gr. doses in afternoon and evening.	Passed blackwater at 1 a.m.
C	(1) 10 grs. a day for two days.	Passed blackwater on the third day.
	(2) (A year later) 10 grs. at 6 a.m.	Passed blackwater at 7 or 8 a.m.
D	(1) 10 grs.	Passed blackwater soon afterwards.
	(2) 5 grs. hypodermically.	Passed blackwater one and a half hours later.
E	10 grs. in evening.	Passed blackwater next morning.
F	6 grs. before and 6 grs. after dinner.	Passed blackwater in night.
G	Three 5-gr. doses of quinine.	After the first dose passed brown urine.
H	5 grs. of quinine a day for two days. Took no more medicine.	Blackwater on the fifth day.
I	10 grs. for two evenings.	Blackwater second night.
J	6 grs. hypodermically at 10 a.m.	Urine porter coloured at mid-day.
K	10 grs.	Blackwater next day.
L	35 grs. in 5-gr. doses in two days.	Blackwater on third day.
M	9 grs. on two successive mornings.	Urine dark on second morning, porter coloured same evening.

medicinally, and as, moreover, quinine is not stored up in the body as such,* it follows that the maximum amount taken medicinally, rarely reaching 3 grammes (46·5 grains), cannot produce haemolysis of healthy red blood cells in the circulation. The possibility of the haemoglobinuria of blackwater fever being due to the direct action of quinine on healthy red cells in the blood stream is thus absolutely excluded. That some other explanation of the action of quinine in causing blackwater fever must be sought is evident also from the smallness of the dose which ordinarily is followed by blackwater. Thus in Table 22, in which thirteen attacks are recorded, five grains or more hypodermically and nine grains or more by the mouth were followed by blackwater fever. These may be taken as representative amounts required to produce blackwater. In animals the hypodermic administration of quinine fails to produce haemoglobinuria, as the experiments recorded in Table 23 show, even when the dose is relatively far higher than in Table 22.

TABLE 23. Haemoglobinuria in relation to quinine in animals.

No. of Experiment	Animal	Amount of quinine bihydrochloride injected per kilo of body weight	Result
1	Rabbit ...	0·13 g. intravenously	Death within an hour.
2	,,	0·08 g. hypodermically	No haemoglobinuria. No ill effect.
3	,,	0·16 g. hypodermically	No haemoglobinuria. No ill effect.
4	,,	0·5 g. hypodermically	No haemoglobinuria. Convulsions at end of 1½ hours; death at end of 2 hours.
5	Dog ...	0·25 g. hypodermically 0·25 g. hypodermically 4¾ hours later	No haemoglobinuria. Unsteady after first injection; convulsions and death ten minutes after second injection.
6	,,	0·15 g. hypodermically	No haemoglobinuria. No ill effect.
7	,,	0·2 g. hypodermically	No haemoglobinuria; no haemoglobinaemia Death at end of one hour.

The fate of quinine in the body is only partially known. Kleine,† Mariani,‡ Schmitz§ and Giemsa and Schaumann¶ showed that the amount of quinine eliminated as such in the urine during the first twenty-four hours after administration is usually 15 per cent. to 26 per cent. of the amount administered. In the body only traces of quinine can be recognised. Giemsa and Schaumann[*] found quinine in the blood in only one out of three cases after its administration, and in this the quinine, which was present in amount too small for estimation, was found in the plasma only, not in the red cells. It would therefore appear that the amount of quinine reaching the blood stream and able to act directly upon the red cells is extremely small.

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* Loc. cit.

† Über die Resorption von Chininsalzen, Zeitschr. . Hygiene u. Infektionskrankheiten. Bd. 38, S. 190.

‡ L'assorbimente e l'eliminazione della chinina e dei suoi sali. Atti della Società per gli studi della malaria, 1904.

§ Über die Ausscheidung des Chinins im menschlichen Harn. Archiv. f. experimentelle Pathologie und Pharmakologie, 1907.

¶ Loc. cit.

(*b*) *The action of quinine on red cells during blackwater fever.*

In the preceding subsection the action of quinine upon healthy red blood cells has been studied in considerable detail, so that the behaviour towards quinine of red blood cells during blackwater fever may be the more readily compared with their behaviour in respect of this alkaloid during health.[†]

Method. The method of testing employed has been that used in Table 1, and involves the withdrawal of about ten drops (0·5 c.cm.) of blood from the finger, the procedure adopted being as follows: The blood coming from the finger was allowed to fall drop by drop into a small collecting capsule of glass, containing a measured amount of a 1 per cent. solution of potassium oxalate. As soon as enough blood had been collected the volume of the mixture was measured. The object of these two measurements was to ascertain the percentage by volume of red cells, and also the haemoglobinometer reading of the undiluted blood. If, however, it is not desired to obtain these data, then the two measurements in question may be omitted. The next step was to make a haemocrit determination of the percentage by volume of red cells in the oxalated blood. As soon as this had been done a measured amount of the oxalated blood was taken up in a pipette and transferred to a centrifugal tube containing about 10 c.cm. of a 0·9 per cent. solution of sodium chloride. After completely precipitating the red cells by centrifugalisation, the supernatant liquid was removed by means of a glass tube provided with a rubber teat and drawn out to a capillary end. To the red cells 0·9 per cent. sodium chloride solution was added in such amount as to produce a 2·5 per cent. emulsion of the red cells. A sample of this emulsion was then diluted with nineteen parts of distilled water and a haemoglobinometer reading made. A series of test tubes was next prepared to which the respective quantities of 0·9 per cent. sodium chloride solution and of 1·92 per cent. quinine bihydrochloride solution given in Table 24 were added. The requisite amounts of the 2·5 per cent. emulsion of red cells were added, and the tubes placed in an incubator kept at 37° C., being subsequently stirred up with a glass rod every fifteen minutes until the conclusion of the experiment at the end of three hours.

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[*] Loc. cit. p. 32.

[†] A. Murri, Sull' intossicasione da chinino, Il Policlinico, 1895, Sezione Medica. Vol. 2, p. 340, tested the action of quinine in varying dilutions on the red blood cells of a patient suffering from haemoglobinuric fever following quinine, and on healthy red blood cells during similar conditions, but no difference was observable in the two cases. No details are given (p. 349).

In those experiments in which complete haemolysis did not occur the degree of haemolysis was indicated by the expressions 'partial,' 'slight,' 'trace.'

In carrying out these experiments it must be borne in mind that the emulsion should contain a definite weight of red blood cells. It cannot be made up with a definite weight of blood, for such a procedure would give no clue as to its real composition in respect of red cells, since it has been found that in health slight, and in blackwater fever and malaria considerable, variations in the relation which the volume of red cells bears to that of the plasma may occur. Nor is it of any practical utility for our purpose to determine the number of red cells per cubic millimetre of blood, since this also gives no measure of their percentage by weight. Nor again is it of advantage to know the red cell index unless the percentage by volume is also known, and when the latter has been determined the former is not required. On the other hand, in order that the conditions of experiment may be more fully defined, it is desirable that the determination of the concentration of red cells by weight in the emulsion employed should be supplemented by a determination of the percentage of haemoglobin given by the reading of a haemoglobinometer standardised as already described. When the two determinations are nearly the same, no difficulty is experienced in comparing the haemolysis produced by quinine with that obtaining in health. When, however, a marked variation occurs, as in Experiments 10, 14a and

TABLE 24. The haemolytic action of quinine bihydrochloride upon red blood cells during and after blackwater fever and malaria. Experiments conducted at 37° C. as in Table 1 (p. 15). Duration of experiment three hours.

No. of Observation	Case of Blackwater Fever	Condition present	Haemoglobin in wet red cells : volume of same.	COMPOSITION OF MIXTURE OF RED CELLS AND QUININE SOLUTION							
				0·9 % NaCl ...	1·00	1·20	1·50	1·80	2·30	2·80	3·20 c.c.
				1·92 % Q. 2 HCl	0·05	0·05	0·05	0·05	0·05	0·05	0·05 "
				2·5 % emulsion of wet red cells	0·20	0·25	0·325	0·40	0·525	0·65	0·75 "
				Concentration of Q. 2 HCl ...	0·080	0·065	0·0535	0·045	0·038	0·029	0·024 %
				Weight of wet red cells ... Weight of Q. 2 HCl ...	5·2 1	6·5 1	8·5 1	10·4 1	13·7 1	16·9 1	18·2 1
1	3	During haemoglobinuria	0·91		Complete	Complete	Complete	Complete (in 2½ hrs.)	—	—	—
2	"	At end of attack of haemoglobinuria	0·91		Complete	Complete	Complete	Complete	Almost complete	Trace	Nil
3	"	A week after the disappearance of blackwater ...	1·11		Complete	Complete	Complete	Trace	Nil	Nil	Nil
4	4	Two days after relapse of haemoglobinuria ...	0·95		—	—	Partial	Almost complete	Trace	—	—
5	5	At end of attack of haemoglobinuria	1·05		—	Complete	—	—	Partial	—	—
6	7	During blackwater	1·18		Complete	—	Complete (in 1½ hrs.)	—	Partial	—	—
7	7a	During haemoglobinuria	1·17		Complete	Complete	Complete	Marked	Trace	Trace	—
8	8	During haemoglobinuria	—		—	Complete	Complete	Complete	Almost complete	Slight	Nil
9	"	At end of attack of haemoglobinuria	—		—	Complete	Complete	Complete	Slight	Trace	Nil
10	9	Four days after attack of haemoglobinuria had ceased	1·36		—	Complete	Complete	Complete	Trace	—	—
11	10	During haemoglobinuria	1·01		Complete	Complete	Complete	Complete	Trace	Nil	Nil
12	"	During relapse of haemoglobinuria	1·18		—	Complete	Complete	Marked	Trace	—	—
13	11	Towards close of attack of haemoglobinuria ...			—	Complete	Complete	Complete	Trace	—	—
14	"	Three days after attack of haemoglobinuria (during partial suppression)			Complete	Complete	Complete	Complete	Trace	Nil	Nil
15	14	Ten hours after end of attack of haemoglobinuria...	—		—	Complete	Complete	Complete	Trace	Nil	—
16	14a	Towards close of attack of haemoglobinuria ...	1·37		—	—	—	—	—	—	—
17	15	During haemoglobinuria	—		—	Complete	Complete	Complete	Trace	Nil	--
18	16	At end of attack of haemoglobinuria	—		—	—	Complete	Complete	Partial	Trace	—
19	—	Malarial attack, temperature normal, parasites numerous	1·24		Complete	Complete	Complete	Slight	Trace	Nil	—
20	—	Malarial attack, temperature 103 (twenty-four hours later parasites few)	1·12		Complete	Complete	Complete	Slight	Trace	Nil	

19, Table 24, in which the ratio of the two determinations is respectively 1·36 : 1, 1·37 : 1 and 1·24 : 1, then a close comparison becomes difficult, since it is no longer possible to state the ratio of the red cells to quinine so precisely as could be desired. It is therefore evident that an extremely close comparison of the action of quinine on red cells in health and in blackwater fever cannot be made. The series of increments adopted in Tables 1 and 24 appear to be the smallest which can conveniently be chosen. Blackwater fever patients are frequently anaemic, and it will be noticed that the haemoglobin of the red cells was generally altered in the direction of an increase in the amount per unit volume of red cells. In Experiment 4, Table 24, although the direct haemoglobinometer reading of the blood was 24, the normal reading being 100, this ratio was scarcely affected, being 0·95.

TABLE 1. Haemolysis of red blood cells by quinine bihydrochloride, dissolved in 0·9 per cent. NaCl solution. Duration of experiment three hours. Temperature 37° C.

No. of Experiment	COMPOSITION OF MIXTURE OF RED BLOOD CELLS AND QUININE SOLUTION.				
	Quinine bihydrochloride 0·080 % $\frac{\text{Weight of wet red blood cells}}{\text{Weight of quinine salt}}$ $\frac{5·2}{1}$	0·065 % $\frac{6·5}{1}$	0·054 % $\frac{36·5}{1}$	0·045 % $\frac{10·4}{1}$	0·038 % $\frac{13·7}{1}$
1	Complete	Complete	Complete	Complete	Partial
2	Complete	Complete	Complete	Complete	Marked
3	Complete	Complete	Complete	Complete	Partial
4	Complete	Complete	Complete	Complete	Partial
5	Complete	Complete	Complete	Complete	Partial

In Table 24 the result of eighteen observations of the haemolysis of red cells by quinine in blackwater fever, and two observations in malaria, is given. For the sake of easy reference, Table 1 is repeated. In the latter Table the transition point is well defined, and is seen to be reached with a concentration of 0·045 per cent. of quinine bihydrochloride and a ratio of red cells (by weight) to quinine bihydrochloride of 10·4 : 1. In Table 24 the same result is obtained in nearly every case, the exceptions being Experiments 3, 4, 12, 19 and 20, in all of which haemolysis proceeded at a slightly slower rate than usual. Since occasional variations are also met with under normal conditions, (cp. Tables 2, 3, 5, 6, 7, 9, 11), it follows that within the limits of accuracy of the method employed no decided change in the susceptibility of the red cells to the action of quinine is recognisable.

The same conclusion also holds if the conditions present are examined individually. During the period of haemoglobinuria eight cases were examined (1, 6, 7, 8, 11, 12, 13, 17); in five of these haemolysis was complete in three hours, the concentration of quinine bihydrochloride being 0·045 per cent.; in two (7, 12) haemolysis proceeded somewhat more slowly; and in one (6) the number of observations was small, but the rate of haemolysis was probably the same as that of the first five. Of the nine cases of blackwater fever examined when haemoglobinuria had ceased (2, 3, 4, 5, 9, 10, 14, 15, 18) six followed the normal course, in two (3, 4) haemolysis proceeded somewhat more slowly than usual, and the remaining one appeared to have followed the usual course, though the number of observations is incomplete.

Two observations were made during a malarial attack (19, 20), one during the apyrexial interval and one during the attack, when the patient's temperature was 103° F. In both of these the haemolysis proceeded at a slightly slower rate than usual, being completed in three hours with a concentration of quinine bihydrochloride of 0·0535 per cent, instead of the more usual concentration of 0·045 per cent. It cannot, however, be inferred from these two observations that a slight diminution in the rate of haemolysis is a constant occurrence, since a similar range of variation may be met with under normal conditions, as already mentioned.

Most of the thirteen attacks of blackwater fever referred to in Table 24 occurred in individuals who were regarded as also affected with malaria, since in all more or less typical malarial attacks preceded the paroxysm of blackwater fever, as a study of the clinical histories given on pp. 176 to 246 will show. Such evidence is, with one exception however, clinical only. In all cases quinine had been administered, and examination of blood films made at the time of blackwater failed to reveal the presence of malarial parasites in any case. The absence during blackwater fever of any obvious alteration in the vulnerability of the red blood cells towards quinine lends, however, further support to the view that no marked change in this respect is to be anticipated in malaria.

The circumstance, brought out quite conclusively in Table 24, that no considerable change in the red blood cells as regards the action of quinine occurs during blackwater fever, is a point of considerable importance, for a moment's consideration will show that, since healthy red cells are not haemolysed by even the greatest concentrations of quinine which may conceivably be reached in the blood when quinine is taken in the doses ordinarily administered in malaria, namely, one-third of a gramme to one gramme (five to fifteen and a half grains), therefore, unless the sensibility of the red cells is enormously increased in blackwater fever, no haemolysis due to the action of quinine on red cells contained in the circulating blood can possibly take place. The circumstance that, in point of fact, no marked change does occur, is therefore conclusive evidence against the action of quinine in blackwater fever being in any way related to a greater vulnerability of the red blood cells taken as a whole. The observations given in Table 24 do not afford any information as to whether any very small fraction of the red blood cells—such as would, owing to its relative insignificance, fail to be recognised by the method employed—may not be unduly sensitive to the action of quinine. In the next sub-section, an observation on the action of quinine on red blood cells containing malarial parasites is recorded, but the further consideration of this problem, which depends for its solution partly upon a knowledge of the actual amounts of red blood cells destroyed during attacks of blackwater fever, will be more conveniently deferred for the present, and dealt with again in Section 5 (pp. 136 to 165).

(c) *The action of quinine on red blood cells affected with malarial parasites.*

In any attempt to elucidate the mechanism of blackwater fever, it is a matter of the first importance to ascertain whether red blood cells affected with malarial parasites are more readily haemolysed by quinine than are healthy red cells. In order to study this point, it is necessary to obtain blood which is relatively rich in intracorpuscular parasites. Nearly all the cases of malaria which came under our notice, however, had already been treated with quinine, and parasites were found in the blood films examined in very scanty numbers or could not be detected after prolonged search. Such cases were, therefore, of no value in settling the enquiry in question. Only a single case occurred in which we were able to test the action of quinine on red cells containing malarial parasites. In this case, on which Observations 19 and 20, Table 24, were made, a count of the red cells showed that about 3 per cent. contained parasites.

Method. The action of quinine was studied in the manner employed in the observations recorded in Table 1. Blood obtained by pricking the finger was allowed to fall drop by drop into a glass capsule containing 0·15 c.cm. of a 1 per cent. solution of potassium oxalate until about 1 c.cm. had been collected, blood smears being made at the same time. The total volume of the oxalated blood was then measured, and a haemocrit estimation made. The red cells were separated by centrifugalisation, washed and then added to 0·9 per cent. solution of sodium chloride in amount required to produce a 2·5 per cent. emulsion. This was mixed with 1·92 per cent. solution of quinine bihydrochloride and 0·9 per cent. solution of sodium chloride in the amounts given in Table 24. The mixtures, after stirring with a capillary glass rod, were kept in a water bath at 37° C. for three hours, when smears were made from the red cells contained in the last two tubes. The supernatant liquid after centrifugalisation was found to be colourless in the one tube, and to be of an exceedingly slight reddish tint in the other.

The result was indecisive. At the close of the experiment the number of parasites contained in red blood cells was diminished, and those present did not stain well. In addition a few free parasites were seen. It would thus appear that the action of the quinine salt was directed towards the parasites, the staining reaction of which was in consequence altered, while many disappeared or became unrecognisable. Some of the red blood cells which contained parasites appeared, therefore, to have been completely destroyed by the action of the quinine salt, for no red blood cells could be seen, which exhibited a partial defect of substance. All the red blood cells showed, however, slightly defective staining reaction, attributable to the action of quinine.

Unfortunately, we were able to make only a single experiment. The problem at issue cannot be settled until a number of such observations have been made. In the meantime, we have thought it worth while to refer to the line of experiment followed.

(d) The action of urine on red blood cells and on dissolved haemoglobin.

In studying the mechanism of blackwater fever, it is essential to ascertain how far the condition of the urine when voided represents its original condition when issuing from the collecting tubules of the kidney, and how far it has been changed while retained in the bladder. This enquiry resolves itself into a two-fold one: into the action of urine upon haemoglobin contained in the red blood cells; and into the action of urine upon laked haemoglobin.

When a solution of haemoglobin is added to urine in amount sufficient to produce a light red coloration, it is found that the red tint soon becomes fainter, the urine turning brown in colour, and that ultimately the whole of the haemoglobin is broken up, a brown soluble pigment remaining, which gives no absorption bands on spectroscopic examination. Sometimes a variable amount of a brown precipitate appears at the end of twenty-four to forty-eight hours, and the urine may then become less brown in colour.

If an emulsion of red blood cells in 0·9 per cent. solution of sodium chloride is added to urine, it is seen that the latter, when the changes in the red cells described below, become advanced, gradually turns brown in colour, but no reddish tint is noticeable. Urine does not produce visible laking of red blood cells unless its specific gravity is less than 1·009. Urine of specific gravity 1·002 lakes red blood cells as rapidly as distilled water. Red blood cells in urine whose specific gravity exceeds 1·009 become, on standing, poorer in haemoglobin and assume a darker somewhat brownish aspect, but are ultimately decolourised and form a brownish-white precipitate at the bottom of the tube in which the urine is contained. If such urine at any time after adding the red blood cells is centrifugalised and the supernatant fluid examined spectroscopically, it is found that no oxyhaemoglobin bands are at first seen, the red blood cells becoming decolourised without any laking of their haemoglobin. When, however, the red blood cells have lost 75 per cent. or more of their haemoglobin, oxyhaemoglobin bands make their appearance in the urine, which is now brownish in colour (cp. Table 23). Just as occurs when dissolved haemoglobin is added to urine, a precipitate brown in colour may make its appearance at the end of twenty-four hours in the mixture of urine and red cells. It is thus seen that in the red cell the haemoglobin is broken up, by urine of specific gravity exceeding 1·009, in sitû, and does not leave the cell until it has become completely converted into brown pigment.

In order that some idea may be obtained of the degree to which haemoglobin is likely to be broken up in the urine in cases of blackwater fever, and, further, in order to determine to what extent small quantities of haemoglobin passing into the urine at the time of its secretion by the kidneys may be recognisable in the urine when voided, the experiments given in Table 25 were carried out. These fall into two series, conducted simultaneously with the same urine and with haemoglobin derived from a single source.

Method. In the first series of experiments human blood cells were added to the urine in such amounts that an emulsion containing from 5 per cent. to 0·33 per cent. of red blood cells was obtained. At the end of four hours at a temperature of 37° C., during which period the red blood cells were distributed in the urine by stirring with a glass rod every fifteen minutes, the mixture was centrifugalised, the supernatant fluid poured off and examined spectroscopically, while the red cells were mixed with a measured quantity of distilled water and, after laking, the amount of haemoglobin still remaining determined by the haemoglobinometer. In the second series the same amounts of haemoglobin were used as in the first, but the haemoglobin instead of being contained in red cells was in solution. In this second series, owing to the formation, especially in the weaker dilutions, of the brownish pigment already referred to, accurate haemoglobinometer readings of the amounts of haemoglobin in the urine could not be made, and instead the oxyhaemoglobin bands present were matched by the aid of a comparison spectroscope with those of a haemoglobin solution of known concentration, and in this way the concentration of haemoglobin in the urine was determined.

On comparing the two series, it is seen that the degree of destruction of haemoglobin proceeds in both very nearly at the same rate, being somewhat more rapid when the haemoglobin is in solution than when it is contained in the red blood cells, but the difference is

TABLE 25. The action of urine in breaking up haemoglobin. Duration of experiment four hours. Temperature 37° C.

No. of Experiment	COMPOSITION OF MIXTURE OF URINE AND RED CELLS						COMPOSITION OF MIXTURE OF URINE AND DISSOLVED HAEMOGLOBIN						CONTROL TUBE	REMARKS
	Amount of urine ...	0·80 c.c.	1·80 c.c.	4·80 c.c.	9·80 c.c.	14·80 c.c.	Amount of urine ...	0·80 c.c.	1·80 c.c.	4·80 c.c.	9·80 c.c.	14·80 c.c.	—	
	Amount of red cells ...	0·05 c.c.	0·05 c.c.	0·05 c.c.	0·05 c.c.	0·05 c.c.	Amount of dissolved haemoglobin ...	0·05 c.c.	0·05 c.c.	0·05 c.c.	0·05 c.c.	0·05 c.c.	0·05 c.c.	
	Amount of 0·9 % NaCl solution ...	0·15 c.c.	0·15 c.c.	0·15 c.c.	0·15 c.c.	0·15 c.c.	Amount of 0·9 % NaCl solution ...	0·15 c.c.	0·15 c.c.	0·15 c.c.	0·15 c.c.	0·15 c.c.	9·95 c.c.	
	Concentration of urine in mixture ...	80 %	90 %	96 %	98 %	98·7 %	Concentration of urine in mixture ...	80 %	90 %	96 %	98 %	98·7 %	—	
	Weight of wet red cells / Weight of urine ...	1/16	1/36	1/96	1/196	1/294	Weight of haemoglobin / Weight of urine ...	1/16	1/36	1/96	1/196	1/294	—	
	Percentage of wet red cells ...	5 %	2·5 %	1 %	0·5 %	0·33 %	Percentage of haemoglobin ...	5 %	2·5 %	1 %	0·5 %	0·33 %	—	
1	Amount of haemoglobin remaining in red cells at end of experiment	65 %	51·5 %	33 %	22·5 %	Too small for measurement	Amount of haemoglobin remaining at the end of experiment ...	—	—	—	—	—	0·524 %	Sp. gr. of urine 1·025. Acid. No albumin.
	Amount of haemoglobin destroyed ...	35 %	48·5 %	67 %	77·5 %	--	Amount of haemoglobin destroyed ...	—	—	—	—	—	—	
2	Amount of haemoglobin remaining in red cells at end of experiment	52·5 %	39 %	32 %	—	—	Amount of haemoglobin remaining at the end of experiment ...	—	37 %	32 %	--	—	0·556 %	Sp. gr. of urine 1·018. Acid No albumin.
	Amount of haemoglobin destroyed ...	47·5 %	61 %	68 %	—	—	Amount of haemoglobin destroyed ...	—	63 %	68 %	—	—	—	
3	Amount of haemoglobin remaining in red cells at end of experiment	60 %	42 %	39 %	29 %	11 %	Amount of haemoglobin remaining at the end of experiment ...	42 %	36 %	28 %	26 %	11 %	0·556 %	Sp. gr. of urine 1·020. Acid. No albumin.
	Amount of haemoglobin destroyed ...	40 %	58 %	61 %	71 %	89 %	Amount of haemoglobin destroyed ...	58 %	64 %	72 %	74 %	89 %	—	
4	Amount of haemoglobin remaining in red cells at end of experiment	60 %	54 %	42 %	26 %	13 %	Amount of haemoglobin remaining at the end of experiment ...	51 %	51 %	42 %	29 %	13 %	—	Sp. gr. of urine 1·015. Faintly Acid. No albumin.
	Amount of haemoglobin destroyed ...	40 %	46 %	58 %	74 %	87 %	Amount of haemoglobin destroyed ...	49 %	49 %	58 %	71 %	87 %	—	

not great. As might be expected, the destruction is relatively smallest when the concentration of the red blood cells, or of the laked haemoglobin, is greatest, and when the concentration of haemoglobin is very low the destruction may be very nearly complete within the period of experiment. The actual figures given will enable some idea to be formed of the degree to which in blackwater fever the amount of haemoglobin present at the end of four hours represents the amount originally present in the urine when first excreted. Thus when the urine originally contained 5 per cent. of red blood cells, the percentage at the end of four hours was only 2·7 per cent. to 3·25 per cent., 35 per cent. to 47 per cent. of the haemoglobin being destroyed. When, however, the urine originally contained 0·33 per cent. of haemoglobin, about 90 per cent. was destroyed, the percentage being reduced to 0·03, a quantity which is not easily recognised or estimated. The percentages in the Table were for the most part ascertained by means of haemoglobinometer readings. The original concentrations were made up by means of the haemocrit, and one of these, as a control, was measured by the haemoglobinometer, the reading obtained being a little higher (0·5 per cent.) than the amount given by the haemocrit.

When the percentage of haemoglobin, whether in the form of red blood cells or of dissolved haemoglobin, was less than 0·01 per cent. in the urine, at the end of four hours it became difficult or impossible to recognise oxyhaemoglobin bands. In these cases the urine, which, as already mentioned, had acquired a brownish tint, gave, on slightly acidifying and heating, a brownish white precipitate of coagulable protein, this forming in many cases the only indication of the original addition of haemoglobin.

Owing to the circumstance that urine is not a fluid of constant composition, it is not possible to calculate, from the amount present at the end of four hours, how much was originally present. In each individual case a special estimation is required. Only an approximate idea of the limits of the range of destruction in different concentrations is afforded by Table 25, which, however, shows that with a high specific gravity of the urine the rate at which destruction proceeds is increased. Destruction of haemoglobin was usually attended with the production of methaemoglobin, which was, however, present only in relatively very small amount. This occurred both within the red cells, and when dissolved haemoglobin was used.

With a view of throwing light upon the nature of the process of destruction of haemoglobin by urine, the rate at which destruction proceeded was investigated in the case of red cells (Table 26). The determination of the rate of destruction, in urine, of dissolved haemoglobin was not attempted owing to the difficulty of obtaining, especially with the higher degrees of destruction, sufficiently readily and accurately estimations of haemoglobin in the presence of the colouring matter of urine.

Method. The mode of conducting these experiments was similar to that adopted in the case of quinine (Tables 17, 18 and 19). Four experiments were carried out with different specimens of healthy urine obtained from the same individual, about three hundred cubic centimetres being taken for each experiment. To this, healthy human red blood cells were added to the extent of 0·488 per cent., 0·484 per cent., 0·448 per cent. and 0·488 per cent. in Experiments 1 to 4 respectively. The mixture of urine and red blood cells was then divided into three equal parts, which were placed in water baths kept at temperatures of 37° C., 31° C. and 25° C. respectively, and were shaken every fifteen minutes so as to secure uniform distribution of the red blood cells. At intervals of half to one hour samples of the mixture (usually 10 c.cm.) were withdrawn and centrifugalised. The supernatant liquid was examined for oxyhaemoglobin and methaemoglobin bands; the red cells were laked with distilled water, the volume of the solution being made up to that of the sample and the percentage of haemoglobin determined with the haemoglobinometer or, if considerable destruction of haemoglobin and production of brown colouring matter had occurred, matched with a haemoglobin solution of known concentration, by the aid of a comparison spectroscope, as already described.

The urine employed in these four experiments varied in respect of specific gravity and reaction (cp. Table 27). The former ranged from 1·026 to 1·015, while the reaction was acid in Experiment 1, slightly acid in Experiments 2 and 3, and slightly alkaline in the fourth experiment. The urine was perfectly clear and free from precipitate.

TABLE 26. The rate of destruction of haemogoblin in red blood cells by urine at different temperatures.

No. of Experiment	t	Percentage of haemoglobin in red cells	T 37° C $\frac{1}{t_x - t_0} \log \frac{C_0}{C_x}$	Percentage of haemoglobin in red cells calculated from value of K	t	Percentage of haemoglobin in red cells	T 31° C. $\frac{1}{t_x - t_0} \log \frac{C_0}{C_x}$	Percentage of haemoglobin in red cells calculated from value of K	t	Percentage of haemoglobin in red cells	T 25° C. $\frac{1}{t_x - t_0} \log \frac{C_0}{C_x}$	Percentage of haemoglobin in red cells calculated from value of K
1	0 min.	100		100 Value of K	0 min.	100		100	0 min.	100		100
	90 ,,	41	0·0043	41 taken at	90 ,,	72	0·0016	70	120 ,,	90	0·00038*	85
	150 ,,	12·5**	0·0060	24 0·0044	150 ,,	55	0·0017	55	180 ,,	71	0·00081	78 Value of K
	210 ,,	1**	0·0096*	12 (Table 24)	210 ,,	48	0·0015	44 Value of K	240 ,,	60	0·00093	72 taken at
					285 ,,	37	0·0015	33 taken at	285 ,,	58	0·00082	67 0·0006
			0·0051 mean		345 ,,	25	0·0017	26 0·0017	345 ,,	54	0·00078	63 (Table 24)
					405 ,,	18	0·0019	20	405 ,,	44	0·00084	57
					525 ,,	9**	0·0020	13	525 ,,	44	0·00064	48
							0·0017 mean				0·00080 mean	
2	0 min.	100		100	0 min.	100		100	0 min.	100		100
	30 ,,	90	0·0015*	85	30 ,,	99	0·0001*	93	165 ,,	91	0·00025*	87 Value of K
	60 ,,	81	0·0015	70 Value of K	60 ,,	97	0·0002*	86	225 ,,	86	0·00031	83 taken at
	90 ,,	66	0·0017	61 taken at	90 ,,	88	0·0005*	80	285 ,,	81	0·00035	79 0·00037
	150 ,,	45	0·0022	44 0·0024	120 ,,	82	0·0007	76 Value of K	345 ,,	71	0·00043	74 (Table 24)
	180 ,,	38	0·0023	37 (Table 24)	150 ,,	74	0·0009	69 taken at	405 ,,	67	0·00043	71
	240 ,,	27	0·0022	27	180 ,,	70	0·0009	63 0·0011				
	300 ,,	18	0·0025	19	240 ,,	48	0·0013	55			0·00038 mean	
					300 ,,	38	0·0014	47				
			0·0021 mean		345 ,,	31	0·0012	41				
					405 ,,	29	0·0013	36				
							0·0011 mean					
3	0 min.	100		100 Value of K	0 min.	100		100	0 min.	100		100
	60 ,,	64	0·0032	58 taken at	60 ,,	81	0·0016	75	135 ,,	83	0·00064	76 Value of K
	120 ,,	23**	0·0052	35 0·0039	120 ,,	62	0·0016	53 Value of K	195 ,,	75	0·00065	67 taken at
	180 ,,	16**	0·0044	20 (Table 24	180 ,,	49	0·0017	41 taken at	255 ,,	58	0·00091	60 0·00088
					240 ,,	23**	0·0026	31 0·0021	315 ,,	53	0·00082	53 (Table 24)
	240 ,,		0·0043 mean		315 ,,	14**	0·0027	22	375 ,,	46	0·00089	47
	315 ,,											
	375 ,,						0·0021 mean				0·00079 mean	
4	0 min.	100		100	0 min.	100		100	0 min.	100		100
	60 ,,	96	0·00028	96	60 ,,	98	0·00013	98	135 ,,	99	0·00001*	98
	120 ,,	93	0·00027	93	120 ,,	97	0·00010	95	195 ,,	96	0·00011	96 Value of K
	180 ,,	88	0·00032	90 Value of K	180 ,,	93	0·00018	93	255 ,,	94	0·0001[illegible]	94 taken at
	240 ,,	78	0·00044	86 taken at	240 ,,	92	0·00014	91 Value of K	315 ,,	91	0·00012	93 0·000092
	300 ,,	77	0·00039	84 0·00026	300 ,,	86	0·00021	89 taken at	375 ,,	[illegible]	0·00012	92 (Table 24)

The destruction of haemoglobin proceeded rapidly at 37° C., usually half to three-quarters disappearing in the course of two hours. At 31° C. one-fifth to two-fifths of the haemoglobin was destroyed in the same period of time, and at 25° C. less than one-tenth. When a comparison of these amounts with the specific gravity and reaction of the urine is made (Table 25), it is seen that with the higher specific gravity (1·026) obtaining in Experiment 1 the rate of destruction was most rapid, with the lower specific gravity of Experiments 2 and 3 (1·020 and 1·022) was less rapid, while in Experiment 4 in which the urine had a specific gravity of 1·015, haemoglobin was destroyed with considerable slowness. In the last case another factor was perhaps operating to retard the action of the urine upon haemoglobin, namely, its alkaline reaction; for alkaline urine was found to be slow in its action upon red cells, even when its specific gravity exceeded 1·020. The variations met with in different experiments seem, however, to be in part attributable to the diurnal variations in the red blood cells already encountered in experiments made with quinine (Tables 16 and 17). The breaking up of haemoglobin in the urine is not due to the presence in the latter of a thermolabile ferment, for previous boiling does not alter the rate at which destruction proceeds.

In red cells, whose haemoglobin has been largely destroyed, methaemoglobin is found, on laking, to be present. The amount of this substance is very small relatively to the unaltered haemoglobin, until the destruction exceeds 85 per cent., when its presence is generally readily recognisable, though it is still found in much smaller amount than oxyhaemoglobin.

It will be seen from Table 26 that haemoglobin did not find its way into the urine until the destruction of haemoglobin within the red cells had reached or exceeded 75 per cent. At about this point haemoglobin began to escape, and when the percentage of destruction exceeded 90 per cent., the percentage of haemoglobin in the urine was sometimes 5 per cent. of the amount originally present in the red blood cells, in one case reaching as much as 10 per cent. Thus, although haemolysis did not occur in amount sufficient to produce after centrifugalisation a red coloration of the urine, nevertheless as destruction of haemoglobin approached completion a slight degree of haemolysis took place, causing oxyhaemoglobin bands to make their appearance.

In determining the reaction rate it will be seen that at the highest temperature employed (37° C.) the number of observations obtainable in Experiments 1 and 3, Table 26, was limited, owing partly to the rapidity of destruction of haemoglobin and partly to the escape of haemoglobin from the red cells into the urine surrounding them, in consequence of which the readings given by the laked cells become too low, and cease to represent the actual destruction. In Experiments 1 to 3, determinations of the monomolecular reaction rate constant show little variation so long as the amount of destruction of haemoglobin lies between 20 per cent. and 80 per cent. Below the lower limit destruction proceeds more slowly, while above the upper limit it proceeds more rapidly. The same variation, in greater degree, has already been seen when quinine produces haemolysis in red blood cells (Tables 13 to 16). And as in the latter case, so here, if the reaction rate constant for bimolecular or multimolecular reactions is calculated from the data given, a greater range of variation is met with, so that these types of reaction are excluded from consideration. In Experiment 4, in which the reaction proceeded very slowly, the reaction rate constant exhibited little variation when the destruction exceeded 7 per cent. (93 per cent. being left unaltered), i.e. after the lapse of about two hours. If the percentages of haemoglobin are recalculated from the reaction rate constants (Columns 4, 8 and 12, Table 24), a fair agreement with the percentages actually found will be observed. It follows therefore that, in the heterogeneous system formed by red blood cells suspended in urine, destruction of haemoglobin proceeds at the same rate as a monomolecular reaction, except at the beginning and end of the process, when slight delay and acceleration respectively occur.

The values of K, the monomolecular reaction rate constant, obtained with the different specimens of urine employed, range from 0·0003 to 0·005 at 37° C., from 0·0002 to 0·002 at 31° C., and from 0·0008 to 0·0001 at 25° C. The values obtained in urine of specific gravity 1·015, whose reaction to litmus paper was alkaline, are, it will be noted, much lower than those obtained in acid urine of higher specific gravity.

In Table 27 the value of the constant μ was determined for each of the four specimens of urine employed. The values obtained, ranging from 13,000 to 30,000, increased as the specific gravity increased and the reaction changed from alkaline to acid (to litmus paper). In the last column the values of K are recalculated from the value of μ obtained and from the mean value of K at 31° C.; a fair agreement is observable between the values found and calculated.

TABLE 27. Determination of value of μ from experiments recorded in Table 26.

No. of Experiment	Temperature of experiment	Value of K determined by experiment (Table 26)	Value of μ calculated from formula $\frac{K_2}{K_1} = e^{\frac{\mu}{2}\left(\frac{T_2 - T_1}{T_2 T_1}\right)}$	Condition of urine	Value of K calculated from μ
1	37° C.	0·0051	30,000	Acid; sp. gr. 1·026	0·0044
,,	31° C.	0·0017			0·0017
,,	25° C.	0·0008			0·0006
2	37° C.	0·0021	24,100	Slightly acid; sp. gr. 1·020	0·0024
,,	31° C.	0·0011			0·0011
,,	25° C.	0·00038			0·00037
3	37° C.	0·0043	19,400	Slightly acid; sp. gr. 1·022	0·0039
,,	31° C.	0·0021			0·0021
,,	25° C.	0·00079			0·00088
4	37° C.	0·00032	13,600	Alkaline; sp. gr. 1·015	0·00026
,,	31° C.	0·00017			0·00017
,,	25° C.	0·00013			0·000092

In Table 26 it will be noticed that the percentages of haemoglobin, remaining undestroyed, have been recalculated in columns 5, 9 and 13, from the corresponding values of μ, taken from the last column of Table 27. The difference between the observed and calculated percentages is seen to be inconsiderable, except when destruction of haemoglobin exceeds 80 per cent.; in the latter case the percentages cannot be estimated with much precision owing to the escape of haemoglobin from the red cells. Table 28 is introduced in order to permit a more ready comparison of the values of K, found and calculated, at individual temperatures. The values of μ, obtained in Table 27, enable the lowering of the rate of destruction of haemoglobin, caused by variation of temperature, to be calculated. Thus a reduction of the destruction rate to $\frac{1}{2{\cdot}63}$ takes place if the temperature is lowered from 37° C. to 31° C. when $\mu = 30{,}000$ and to $\frac{1}{1{\cdot}54}$

when $\mu = 13{,}000$; in the former case a reduction of the temperature of 37° C. by one degree leads to a reduction of the rate of destruction from 1 to 0·855, and in the latter case from 1 to 0·925. The same relationship may be stated in another way: the rate of destruction of haemoglobin at 37° C. becomes reduced to one half when the temperature is lowered 5° C. if $\mu = 30{,}000$, and when the temperature is lowered 9° C. if $\mu = 13{,}000$. These values are nearly the same as those obtaining in the actual experiments.

TABLE 28. Comparison of values of K at different temperatures. Cp. Table 27.

T	No. of Experiment				Calculated for $\mu = 30{,}000$	24,100	19,400	13,60
	1	2	3	4	1	2	3	4
37° C.	0·0051	0·0021	0·0043	0·00032	0·0044	0·0024	0·0039	0·00026
31° C.	0·0017	0·0011	0·0021	0·00017	0·0017	0·0011	0·0021	0·00017
25° C.	0·0008	0·00038	0·00079	0·00013	0·0006	0·00037	0·00088	0·000092

It is thus seen that in the presence of urine the rate of destruction of haemoglobin contained in red blood cells resembles that of a monomolecular process, and is in this respect similar to the rate of haemolysis of red blood cells in the presence of quinine in the alkaloidal state (Tables 13 to 19). The two processes are, however, of a different character, the former being apparently a direct action upon haemoglobin, while the latter seems to be the result of an action directed primarily to the stromata of the red blood cells.

SUMMARY

The principal points in the above investigation may be summarised as follows:—

1. Quinine bihydrochloride and quinine in the alkaloidal state produce haemolysis of red blood cells, as do also hydrochloric acid and sodium hydrate.

2. The action of quinine in the alkaloidal state in producing haemolysis resembles a catalytic action.

3. Haemoglobin breaks up at a monomolecular rate under the action of quinine in the alkaloidal state.

4. The above-mentioned four haemolytic agents possess, in equimolecular concentration, nearly the same haemolytic power, quinine in the alkaloidal state being weaker, and quinine bihydrochloride stronger than hydrochloric acid and sodium hydrate, which occupy an intermediate position.

5. Owing to the toxicity of quinine its concentration in the blood cannot reach an amount sufficient to allow of its direct haemolytic action on red cells taking place during life.

6. The red blood cells during blackwater fever are not haemolysed by quinine bihydrochloride more readily than in health.

7. In the presence of urine haemoglobin, whether contained in red blood cells or in solution, is broken up. In the former case this proceeds at a monomolecular rate, no haemoglobin being discharged from the red cells into the urine, until destruction is nearly complete.

8. The constant, μ, for the haemolysis of red blood cells was found to have a mean value of 15,000 for a 0·00113 M solution of alkaloidal quinine. The value of μ for the destruction of haemoglobin in the presence of urine ranged, in the experiments made, from 13,000 to 30,000. The values of K_{37° ranged in the former cases from 0·0026 to 0·0087, and in the latter case from 0·0026 to 0·0039.

II. THE RELATION OF HAEMOLYSINAEMIA TO THE HAEMOGLOBINURIA OF BLACKWATER FEVER.

In no single instance, up to the present time, has the mechanism of production of haemoglobinuria in blackwater fever been established, though assertions respecting its mode of production are constantly to be met with in text-books and in clinical articles. The only form of haemoglobinuria, of which the mechanism is in part known, is paroxysmal haemoglobinuria, and before proceeding further it will be of advantage briefly to recapitulate the facts which have recently been established in connection with the mechanism of production of this condition. In 1904 Donath and Landsteiner* showed that, in the intervals between the attacks of haemoglobinuria, if the blood rendered fluid by potassium oxalate, or a mixture of the patient's serum and the patient's washed red blood cells, was cooled and then warmed to body temperature, a marked haemolysis occurred, which was not observed if the blood was kept at body temperature, without previous cooling. The red blood cells of the patient were found to be normal, and might be replaced by foreign human red cells; only the serum or plasma is changed, and the haemolysin could be extracted in the cold from the serum by red blood cells, which became dissolved when the serum of the patient or normal human serum was added, the reaction being completed by the addition of complement. The minimum time of cooling required in ice cold water was five to ten minutes (in one case two minutes). Cooling to 10° C. for half an hour was followed by partial haemolysis. This condition was not met with in health, nor in patients suffering from any disease other than paroxysmal haemoglobinuria, with the exception of general paralysis, in which affection it was found that in six out of sixty-six cases the same reaction was obtained.

Donath's and Landsteiner's results were confirmed and their conclusions disputed by Widal and Rostaine,† who have arrived at a somewhat different interpretation of the reaction in question. These authors point out that Bordet had demonstrated the existence of 'substance antisensibilisatrice' (anti-immune body) for red blood cells, and Metchnikoff for spermatoxine, while Besredka has shown that red blood cells remain preserved in their own serum in consequence of the inhibitory action of the substance 'antisensibilisatrice' upon the 'sensibilisatrice' usually present. The authors have come to the concluson that the haemolysis in vitro obtained by Donath and Landsteiner is due to the insufficiency of the substance 'antisensibilisatrice.' They appear to base their conclusion upon the effect of an 'antisensibilisatrice' serum, which they prepared, one drop of which inactivated ten drops of the plasma of a patient suffering from paroxysmal haemoglobinuria, to which three drops of human red cells were added. The antiserum was prepared by injecting, three or four times, massive doses of the serum of a haemoglobinuric patient into animals. The authors used this serum with complete success to arrest haemoglobinuria when cold was applied to the patient's hands, but with only partial success when the test was made in vitro.

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* Über paroxysmale Hämoglobinurie. Münch. med. Wochenschr., 1904, No. 36.; Über paroxysmale Hämoglobinurie, Zeitschr. f. klin. Medizin, 1906, Bd. 58, S. 173.

† Insuffisance de l'antisensibilisatrice dans le sang des haemoglobinuriques. Comptes rendus de la Société de Biologie, 1905, T. 1, p. 321; Insuffisance de l'antisensibilisatrice dans le sang d'un haemoglobinurique. ibid., p. 372; Sérothérapie préventive de l'attaque de l'hémoglobinuria paroxystique, ibid., p. 397.

In view of the above facts, we decided to ascertain if the blood plasma in blackwater fever possessed a similar haemolytic property.

Method. Our own experiments were conducted as follows:—The patient's finger, having been cleansed, was pricked and about ten drops (0·5 c.cm.) of blood were allowed to flow into a small collecting tube containing 1 c.cm. of a 1 per cent. solution of potassium oxalate. The oxalated blood was then transferred by means of a fine pointed pipette to a small centrifugal tube. After centrifugalisation the plasma was pipetted off and put into another tube. The red cells were then made up into a 2·5 per cent. emulsion in 0·9 per cent. sodium chloride solution, and of this one part was added to nineteen parts of the oxalated plasma, so that the ratio of the weights of the wet red cells and plasma respectively would be one to seven hundred and fifty. In some of the experiments, as Tables 29 and 30 show, human red cells not taken from the patient were employed. In some of the later experiments also red blood cells were added in such an amount as to produce a thin emulsion, without reference to the exact proportion of red cells present. The tube containing the plasma and red cells was then put into a freezing mixture obtained by adding solid ammonium nitrate to water contained in a beaker, which was placed in a second larger beaker, surrounded by cotton wool and supported on cork. Between the two beakers an air space existed, as shown in Fig. 1. A thermometer dipped into the ammonium nitrate mixture was used for stirring up the solid salt from time to time as required. In this way the temperature of the mixed plasma and red cells was kept at 0° C. to 5° C. If desired a temperature below 0° C. could have been maintained. The time elapsing between collecting and centrifugalising the blood was in most cases about six minutes; that between pipetting off the plasma and cooling it in the freezing mixture was generally about twenty minutes to two hours. While in the freezing mixture the red cells were once or twice distributed in the plasma by stirring with a capillary glass rod. After being kept in the freezing mixture for half an hour, the tube containing the plasma and red cells was put for three hours in an incubator kept at 37° C., the red cells being stirred from time to time with a glass rod as before.

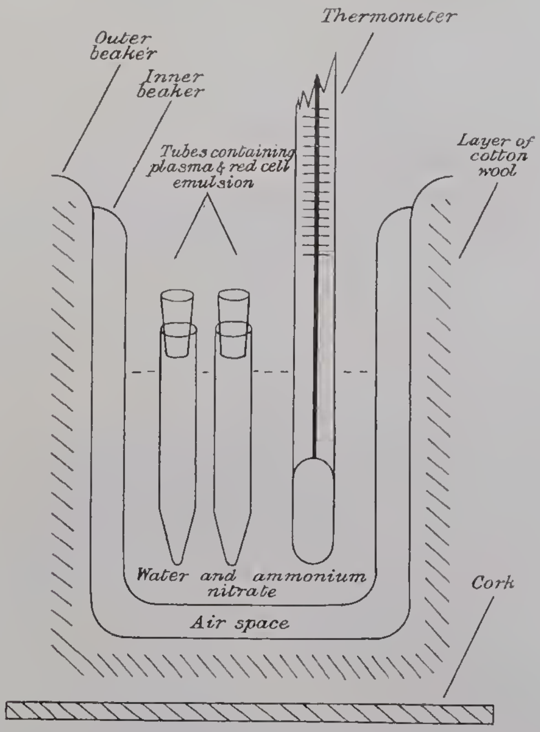
Thermometer
Outer beaker
Inner beaker
Tubes containing plasma & red cell emulsion
Layer of cotton wool
Water and ammonium nitrate
Air space
Cork

FIG. 1. Apparatus employed for testing haemolytic property of blood plasma.

Table 29. Experiments made to ascertain if a haemolytic action on red blood cells could be obtained with the blood plasma of individuals suffering from blackwater fever.

No. of Observation	Case	Condition of subject	Ratio of oxalated plasma to 2·5 % emulsion of red cells	Mixture kept at	For	Then kept at 37° C. for	Result
1	2	Sixteen hours before attack of blackwater	20 : 1	0° C.-4° C.	30 min.	3 hours	No isolysin present in blood plasma
2	,,	Two hours before attack of blackwater	,,	,,	,,	,,	,, ,,
3	,,	During attack of blackwater	,,	,,	,,	,,	,, ,,
4	,,	At end of attack of blackwater ...	,,	,,	,,	,,	,, ,,
5	,,	Six days later	,,	,,	,,	,,	,, ,,
6	3	Twenty hours before attack of haemoglobinuria	,,	,,	,,	,,	No autolysin present in blood plasma
7	,,	During attack of haemoglobinuria ...	,,	,,	,,	,,	,, ,,
8	,,	During attack of haemoglobinuria ...	,,	,,	,,	,,	,, ,,
9	,,	At end of attack of haemoglobinuria...	,,	,,	,,	,,	,, ,,
10	4	Three days after attack of haemoglobinuria	,,	,,	,,	,,	,, ,,
11	,,	Two days after relapse of haemoglobinuria	,,	,,	,,	,,	,, ,,
12	5	Twelve hours after disappearance of haemoglobinuria	,,	,,	,,	,,	,, ,,
13	6	At end of attack of haemoglobinuria	,,	,,	,,	,,	No isolysin present in blood plasma
14	6a	During attack of blackwater	,,	,,	,,	,,	No autolysin present in blood plasma
15	7	During attack of blackwater	,,	,,	,,	,,	,, ,,
16	7a	During attack of haemoglobinuria ...	,,	,,	,,	,,	,, ,,
17	8	During attack of haemoglobinuria ...	,,	,,	,,	,,	,, ,,
18	9	Four days after attack of haemoglobinuria	,,	,,	,,	,,	Complete haemolysis of red cells*
19	,,	Two months later	,,	,,	,,	,,	No autolysin present in blood plasma
20	10	During attack of haemoglobinuria ...	,,	,,	,,	,,	,, ,,
21	,,	During relapse of haemoglobinuria ...	,,	,,	,,	,,	,, ,,
22	11	During attack of haemoglobinuria ...	,,	,,	,,	,,	,, ,,
23	,,	At end of attack of haemoglobinuria (during suppression)	,,	,,	,,	,,	,, ,,
24	,,	Three days later (during suppression)	,,	,,	,,	,,	,, ,,
25	12	At end of attack of haemoglobinuria	,,	,,	,,	,,	Haemolysis of red cells†
26	,,	One day later	,,	,,	,,	,,	No autolysin present in blood plasma
27	14	At end of attack of haemoglobinuria	,,	,,	,,	,,	,, ,,
28	15	During attack of haemoglobinuria ...	,,	,,	,,	,,	,, ,,
29	,,	During attack of haemoglobinuria (during partial suppression)	,,	,,	,,	,,	,, ,,
30	16	At end of attack of haemoglobinuria	,,	,,	,,	,,	,, ,,

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* No turbidity, smell or other indication of putrefaction. Blood plasma examined ten hours after collection. Room temperature 28·5° C.

† Bacteria present in blood plasma, which was examined twenty-four hours after collection.

In Table 29 the result of testing, by the method above described, the plasma in sixteen attacks of blackwater fever, before, during and shortly after the paroxysm, is given, the examination being usually made within an hour of the withdrawal of blood. In addition, twenty-eight other observations upon healthy individuals, and also, for the sake of comparison, upon ten cases of malaria, are recorded in Table 30.

TABLE 30. Experiments made to ascertain if a haemolytic action on red blood cells could be obtained with the blood plasma of individuals suffering from malarial and other affections. Normal plasma is also employed in eleven observations.

No. of Observation	Condition of subject	Ratio of oxalated plasma to 2·5 % emulsion of red cells	Mixture kept at	For	Then kept at 37°C. for	Result
1	Healthy	20 : 1	0°C.-4°C.	30 min.	3 hrs.	No autolysin present in blood plasma
2	,, 4 days later ...	,,	,,	,,	,,	,, ,,
3	,, 10 days later ...	,,	,,	,,	,,	,, ,,
4	Healthy	,,	,,	,,	,,	,, ,,
5	,, 2 days later ...	,,	,,	,,	,,	,, ,,
6	,, 5 days later ...	,,	,,	,,	,,	No isolysin present in blood plasma
7	Healthy	,,	,,	,,	,,	,, ,,
8	Healthy	,,	,,	,,	,,	,, ,,
9	Healthy (Native) ...	,,	,,	,,	,,	,, ,,
10	Healthy	,,	,,	,,	,,	,, ,,
11	Healthy	,,	,,	,,	,,	,, ,,
12	Typhoid	,,	,,	,,	,,	No autolysin present in blood plasma
13	Pyrexia (Native)	,,	,,	,,	,,	No isolysin present in blood plasma
14	Pleurisy (Native) ...	,,	,,	,,	,,	,, ,,
15	Bullous eruption on thighs and legs (Native) ...	,,	,,	,,	,,	,, ,,
16	Pneumonia (Native) ...	,,	,,	,,	,,	,, ,,
17	Malaise (Native)	,,	,,	,,	,,	,, ,,
18	Malaria	,,	,,	,,	,,	No autolysin present in blood plasma
19	Uraemia, T. 106° F. ...	,,	,,	,,	,,	No isolysin present in blood plasma
20	Malaria	,,	,,	,,	,,	Slight haemolysis of red cells *
21	,, next day	,,	,,	,,	,,	No isolysin present in blood plasma
22	Malaria, T. 104° F. ...	,,	,,	,,	,,	,, ,,
23	Malaria (Indian)	,,	,,	,,	,,	,, ,,
24	Bilious fever	,,	,,	,,	,,	,, ,,
25	Malaria	,,	,,	,,	,,	,, ,,
26	Malaria	,,	,,	,,	,,	,, ,,
27	Malaria	,,	,,	,,	,,	,, ,,
28	Malaria	,,	,,	,,	,,	,, ,,

* Partial clotting of blood occurred during collection.

It will be seen that in all cases, with one exception, a negative result was obtained. In the cases of blackwater fever this negative result occurred, not only when the patient's plasma obtained during haemoglobinuria (twelve observations: 3, 7, 8, 14, 15, 16, 17, 20, 21, 22, 28, 29) was used, but also when that obtained both before blackwater appeared (three observations: 1, 2, 6) and (with one exception) shortly after haemoglobinuria had disappeared (nine observations: 4, 9, 10, 12, 13, 23, 25, 27, 30), as also (with one exception) some time after complete recovery had taken place (six observations: 5, 11, 18, 19, 24, 26). From these experiments the important conclusion follows, that the mechanism of production of blackwater stands in an altogether different category from that of paroxysmal haemoglobinuria. The presence of haemolysin or of defect of antilysin in the plasma, which is present in the latter case, is absent in blackwater fever, where, therefore, search must be made for other factors.

Table 30 shows that, as might be expected from the behaviour of the plasma of the blackwater fever patients, who had, in all the cases we examined, been the subjects of malaria, usually having had frequently repeated attacks, a negative result was also obtained in all but one of the cases of malaria dealt with (nine observations, 18, 20, 21, 22, 23, 25, 26, 27, 28).

In three cases a positive result was obtained. The first occurred in a patient suffering from blackwater fever, the plasma being taken four days after the cessation of haemoglobinuria (18, Table 29). When the plasma mixed with the patient's red cells was placed, after cooling for half an hour, in the incubator, slight haemolysis was visible at the end of twenty minutes, and complete haemolysis had occurred at the end of an hour and a half. When an examination of the same patient's plasma was made two months later, when he was in good health, though still troubled with occasional malarial attacks, a negative result was obtained (19, Table 29). The positive result obtained in this case was quite exceptional, and does not affect the general conclusion obtained with the rest of the blackwater fever cases. The second case (25, Table 29) occurred in another patient at the close of haemoglobinuria In this case the examination was made twenty-four hours after collection of blood, and bacteria were present, so that no conclusion as to presence or absence of haemolysin in the patient's blood can be drawn. It may here be observed that the growth of bacteria in oxalated plasma is not necessarily attended with haemolysis of red cells contained in the plasma; in contaminated plasma haemolysis is sometimes observed, but is not unfrequently absent. The third case (25, Table 30) occurred with a malarial patient's blood. Haemolysis was slight, and next day, on repeating the observation, no haemolysis was obtained.

The blood plasma of two oxen suffering from Texas fever was examined, on the day before death, during the passage of redwater. No autolysin was present in either case.

The blood plasma of two dogs suffering from piroplasmosis, both passing urine free from haemoglobin, was also examined. In each case no autolysin was found.

SUMMARY

The haemoglobinuria of blackwater fever is not dependent upon haemolysinaemia.

III. THE RELATION OF HAEMOGLOBINAEMIA TO HAEMOGLOBINURIA IN BLACKWATER FEVER.

In the preceding sections it has been shown that the haemoglobinuria of blackwater fever cannot be attributed to the direct action of quinine on the red cells contained in the blood, nor is it associated ordinarily with the possession of a haemolytic power by the patient's blood plasma. These two possible factors in the mechanism of production of haemoglobinuria having been excluded, the way is clear for the consideration of the relationship of haemoglobinuria to haemoglobinaemia. Upon this point there is no consensus of opinion among writers, nor have different observers obtained concordant results respecting the existence of haemoglobinaemia in blackwater fever. Berthier* and Hymans van der Bergh† were unable to observe red tinted serum during blackwater, though on spectroscopical examination oxyhaemoglobin bands were obtained; A. Plehn‡ found dissolved haemoglobin and also bile pigment in the blood plasma during blackwater; Bignami§ found no dissolved haemoglobin in the blood plasma during blackwater; Murri,¶ who is of opinion that haemoglobin cannot pass through the kidneys until the renal epithelium has undergone changes, states that haemoglobinaemia may persist for hours before haemoglobinuria appears. Stephens‖ states guardedly: 'Not infrequently the serum shows no trace of haemoglobin, although the haemoglobinuria may be actually increasing. Haemoglobinaemia does however occur.' No quantitative determination of the amount of haemoglobin in the blood plasma in blackwater fever appears to have been made by any observer, however, and the views held in this connection appear to have been based entirely upon inspection of the serum. The only experimental work dealing with the relationship in question seems to be that of Ponfick,* who found that in haemoglobinaemia, haemoglobinuria occurred only when the amount of haemoglobin set free in the blood exceeded one-sixtieth of the total amount contained in the red cells. If less were set free, then the activity of the liver was sufficient to convert the haemoglobin into the constituents of the bile, in particular into bile colouring matter, whereby hypercholia, icterus and dark coloration of the urine were produced.

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*Quoted by Marchiafava and Bignami, Malarial Haemoglobinuria, Twentieth Century Practice of Medicine, London, 1900, Vol. 19, p. 483.

† Bydrage tot de kennis der Zwartwaterkorts, Nederl. Tydschrift voor Geneeskunde, 1904.

‡ Ätiologie und Pathologenese des Schwarzwasserfiebers, Virch. Arch., 1903, B. 174, S. 509.

§ Marchiafava and Bignami, loc. cit.

¶Quoted by Marchiafava and Bignami, loc. cit.

‖A System of Medicine. Allbutt and Rolleston, Vol. II, Pt. 2, p. 297, London, 1907.

The reinvestigation of this subject, which it was decided to undertake, includes two distinct enquiries: (1) Is haemoglobinaemia present in blackwater fever? (2) Is haemoglobinuria readily producible as the result of haemoglobinaemia, and, if so, is there any quantitative relationship between the two?

The first enquiry involved the examination of the blood plasma in blackwater fever before, during and shortly after the haemoglobinuria. As control observations the plasma of normal persons, who had never had blackwater fever, was examined, and in order to afford further comparison the plasma of individuals suffering from malaria and other morbid conditions was studied.

Method. The mode of examination was as follows:—The finger, having been previously carefully cleansed and dried, was pricked with a bayonet pointed needle and blood allowed to fall drop by drop into a collecting vessel containing 0·1 c.cm. of a 1 per cent. solution of potassium oxalate, about 0·5 c.cm. (ten drops) of blood being collected. Without any delay the oxalated blood was centrifugalised until the red cells were completely precipitated. The supernatant plasma was pipetted off and transferred to a small glass cylinder 18 mm. high and 4 mm. in internal diameter. This was then placed under a double spectroscope, a cell, the height of which could be varied, filled with a solution of haemoglobin of suitable concentration, being placed in the path of the second spectrum, and the height of the column of the solution it contained altered until the oxyhaemoglobin bands which it produced matched in point of intensity those given by the plasma. The concentration of the haemoglobin solution was determined by means of a haemoglobinometer reading, the absolute values of the scale of the instrument having been determined in terms of the red cells (in the moist condition) of one of us taken as a standard.

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*Virch. Arch., 1875, B. 62, S. 273; Berl. kl. Wochenschr., 1883, No. 26.

When healthy human blood was collected in the manner above described, the plasma was found to be light orange coloured and to present no trace of a red tint in a tube of about 5 mm. internal diameter. If examined by the spectroscope in a column 18 mm. high, oxyhaemoglobin bands were always found, not unfrequently, it is true, on the threshold of visibility. When matched with a solution of haemoglobin, the amount present was represented by percentages of red cells ranging from 0·25 per cent. to 0·10 per cent. or less (Table 31). When the serum is light orange in colour the limit of visibility of the oxyhaemoglobin bands, it may be observed, is in a column 18 mm. high about 0·02 per cent. in daylight of moderate intensity. The haemoglobin present was not due to the red blood cells having been incompletely removed by centrifugalisation, for microscopical examination, aided by further centrifugalisation, failed to reveal the presence of red cells, while in concentrations of 0·1 per cent. or more, due to the presence of red cells, the plasma was obviously turbid to the naked eye, and when examined with a hand lens red cells were readily seen in fair abundance. On the other hand, it is clear that the amount of haemoglobin present is dependent to some extent upon the technique, for if two samples of blood are withdrawn from the finger in succession, the intensity of the bands in the plasma will be found as a rule to exhibit some variation, which is, however,

TABLE 31. Examination of oxalated blood plasma, for the presence of dissolved haemoglobin, in healthy men.

No. of Observation	Condition of subject supplying plasma	Amount of blood : amount of potassium oxalate solution	Colour of oxalated plasma in a layer 3 mm. thick	Length of column examined with spectroscope	Percentage of haemoglobin in the blood plasma
1	Healthy	4 : 1	Orange	18 mm.	0·25 %
2	„ (twelve days later)	„	Orange	„	0·13 %
3	„ (nine months later)	„	Light orange	„	0·15 %
4	Healthy	„	Light orange	„	0·10 %
5	„ (one day later) ...	„	Light orange	„	0·13 %
6	„ (seven days later) ...	„	Light orange	„	0·13 %
7	Healthy	„	Light orange	„	0·10 %

relatively slight in amount. The cause of such variation is not easy to determine. The amount of squeezing to which the finger may be subjected stands in no definite relation to the intensity of the bands present, though mechanical injury to the red cells, as in whipping blood, produces some degree of laking; nor does the length of time (up to one and a half hours) during which the oxalated blood is kept before centrifugalisation ordinarily affect the strength of the bands in any definite manner. The only circumstance which constantly affected the haemoglobin bands was the occurrence of clotting. If a clot formed, the serum expressed always showed stronger haemoglobin bands than the plasma, and if a sterile clot was allowed to stand for three hours the serum in contact with it usually showed in a thickness of 5 mm. to 7 mm., a reddish tint just recognisable with the naked eye. The haemoglobin bands, moreover, are deepest in those portions of the serum, which are last exuded. If the clot is broken up with a glass rod the red coloration becomes deeper.

TABLE 32. Examination of oxalated blood plasma, for the presence of dissolved haemoglobin, in patients not suffering from blackwater fever.

No. of Observation	Condition present in subject supplying plasma	Amount of blood : amount of potassium oxalate solution	Colour of oxalated plasma in a layer 3 mm. thick	Length of column examined with spectroscope	Percentage of haemoglobin in the blood plasma
1	Malaria (Indian)	4 : 1	Light orange	18 mm.	0·31 %
2	T. 101° F.	,,	Light orange	,,	0·13 %
3	Gummata of liver	,,	Light orange	,,	0·13 %
4	Herpes Zoster	,,	Light orange	,,	0·10 %
5	Malaria	,,	Light orange	,,	0·13 %
6	Malaria	,,	Light orange	,,	0·10 %
7	,, (six days later) ...	,,	Light orange	,,	0·10 %
8	,, (seven days later)	,,	Light orange	,,	0·31 %
9	Malaria (Indian)	,,	Light orange	,,	0·06 %
10	Malaria	,,	Light orange	,,	0·13 %
11	Typhoid	,,	Light orange	,,	0·16 %
12	Malaria	,,	Light orange	,,	0·02 %
13	Malaria	,,	Dark orange	,,	0·09 %
14	,, (one day later) ...	,,	Dark orange	,,	0·20 %
15	Diarrhoea	,,	Light orange	,,	0·05 %
16	T. 102° F.	,,	Light orange	,,	0·04 %
17	Dysentery	,,	Orange	,,	0·16 %
18	Dysentery	,,	Light orange	,,	0·04 %
19	Malaria	,,	Light orange	,,	0·15 %
20	Malaria	,,	Orange	,,	0·05 %
21	Malaria	,,	Light orange	,,	0·07 %

TABLE 33. Examination of oxalated blood plasma, for the presence of dissolved haemoglobin, in blackwater fever patients.

No. of Observation	Condition of subject supplying plasma	Amount of blood : amount of potassium oxalate solution	Colour of oxalated plasma in a layer 3 mm. thick	Length of column examined with spectroscope	Percentage of haemoglobin in blood plasma
1	Case 2—Sixteen hours before attack of blackwater	4 : 1	Dark orange	18 mm.	0·13 %
2	,, Two hours before attack of blackwater	,,	Dark orange	,,	0·13 %
3	,, During attack of blackwater	,,	Dark orange	,,	0·13 %
4	,, At end of attack of blackwater... ...	,,	Dark orange	,,	0·13 %
5	Case 3—Twenty hours before attack of haemoglobinuria	,,	Dark orange	,,	0·16 %
6	,, During attack of haemoglobinuria ...	,,	Dark orange with reddish tint	,,	0·57 %
7	,, During attack of haemoglobinuria ...	,,	Dark orange with reddish tint	,,	0·40 %
8	,, At end of attack of haemoglobinuria ...	,,	Orange	,,	0·08 %
9	,, Twenty-four hours later	,,	Orange	,,	0·08 %
10	,, Seven days later	,,	Light orange	,,	0·11 %
11	Case 4—At end of attack of haemoglobinuria ...	,,	Dark orange	,,	0·06 %
12	,, Two days after a relapse of haemoglobinuria	,,	Light orange	,,	0·06 %
13	Case 5—At end of attack of haemoglobinuria ...	,,	Light orange	,,	0·13 %
14	Case 6—At end of attack of haemoglobinuria ...	,,	Dark orange	,,	0·13 %
15	Case 6a—At end of attack of blackwater ...	,,	Light orange	,,	0·16 %
16	Case 7—During attack of blackwater	,,	Dark orange	,,	0·13 %
17	,, During convalescence, three days later	,,	Light orange	,,	0·06 %
18	Case 7a—During attack of haemoglobinuria ...	,,	Dark orange with reddish tint	,,	0·74 %
19	Case 8 During attack of haemoglobinuria ...	,,	Dark orange	,,	0·13 %
20	,, At end of attack of haemoglobinuria ...	,,	Dark orange	,,	0·07 %
21	Case 10—During attack of haemoglobinuria ...	,,	Dark orange with reddish tint	,,	0·85 %
22	,, Towards close of attack of haemoglobinuria	,,	Dark orange	,,	0·09 %
23	,, During slight relapse of haemoglobinuria	,,	Dark orange	,,	0·30 %
24	,, During another slight relapse of haemoglobinuria	,,	Dark orange	,,	0·20 %
25	Case 11—Towards close of attack of haemoglobinuria	,,	Dark orange	,,	0·25 %
26	,, At end of attack of haemoglobinuria (during suppression)	,,	Dark orange	,,	0·16 %
27	,, Three days later (during suppression)...	,,	Light orange	,,	0·10 %
28	Case 12—At end of attack of haemoglobinuria ...	,,	Dark orange	,,	0·20 %
29	,, Next day	,,	Orange	,,	0·14 %
30	Case 14—During attack of haemoglobinuria ...	,,	Light red	,,	0·57 %
31	,, Ten hours after close of attack ...	,,	Orange	,,	0·18 %
32	Case 14a—Towards close of attack of haemoglobinuria	,,	Dark orange	,,	0·20 %
33	Case 15—During attack of haemoglobinuria ...	,,	Dark orange with reddish tint	,,	0·56 %
34	,, During attack of haemoglobinuria ...	,,	Dark orange	,,	0·42 %
35	Case 16—At end of attack of haemoglobinuria ...	,,	Dark orange	,,	0·20 %
36	Case 17—During attack of haemoglobinuria ...	,,	Dark orange with reddish tint	,,	0·65 %
37	,, During attack of haemoglobinuria ...	,,	Dark orange with reddish tint	,,	0·67 %
38	,, During attack of haemoglobinuria ...	,,	Dark orange with reddish tint	,,	0·95 %
39	,, During attack of haemoglobinuria ...	,,	Dark orange with reddish tint	,,	0·48 %
40	,, At end of attack of haemoglobinuria ...	,,	Dark orange	,,	0·18 %

When the blood of individuals, not in normal health, but suffering from malaria or other affections met with in the tropics, was examined it was found that the variations in the amount of haemoglobin dissolved in the blood plasma lay within much the same limits as in health (Table 32). Thus in fourteen cases of malaria the percentage of dissolved haemoglobin in the blood plasma ranged in twelve cases between 0·02 per cent. and 0·20 per cent., but reached in two cases the somewhat high degree of 0·31 per cent. (in neither of the two latter did blackwater fever make its appearance); in the remaining seven cases (dysentery, diarrhoea, etc.) the range was between 0·04 per cent. and 0·16 per cent. It may here be noted that in perfectly healthy rabbits and oxen we have failed to find dissolved haemoglobin in the oxalated blood plasma. Examined in the above described manner it was found that the blood plasma in blackwater fever gave percentages of dissolved haemoglobin ranging from less than 0·06 per cent. to 0·95 per cent. (Table 33). The number of cases examined was seventeen, and the number of estimations made amounted to forty. The observations fall into three classes: those made before, after and during the passage of blackwater. In the first class, which includes only three observations (1, 2, 5), the percentage of dissolved haemoglobin was 0·13 per cent. or less. In the second class, which includes eighteen observations (4, 8, 9, 10, 11, 12, 13, 14, 15, 17, 20, 26, 27, 28, 29, 31, 35, 40), usually made shortly after the close of the attack, the percentage of dissolved haemoglobin was from 0·20 per cent. to 0·06 per cent. In the third class, consisting of nineteen observations given in Table 33 in thick type, the percentage of dissolved haemoglobin was in twelve (6, 7, 18, 21, 23, 30, 33, 34, 36, 37, 38, 39) cases raised lying between 0·95 per cent. and 0·30 per cent.; in the remaining seven (3, 16, 19, 22, 24, 25, 32) observations it lay within the limits met with in health, namely, 0·25 per cent. to 0·09 per cent. With regard to the latter, it must be remarked that although in these observations the first specimen of urine voided after the examination of the blood plasma contained haemoglobin, as will be seen by reference to the clinical histories on pp. 176 to 246; nevertheless it does not follow that the urine which was being excreted at the time of examination of the blood plasma then contained haemoglobin. It is possible, if not probable, that in some cases the haemoglobin had already disappeared, though, owing to the relatively long intervals at which the patient passed urine, this did not become evident. Much variation, it may be observed, was exhibited in respect of the passage of urine, in some attacks urine being passed frequently, in others retained long in the bladder. Failure to observe haemoglobinaemia — assuming for the moment that this is constantly present during haemoglobinuria, a point which will be further considered on pp. 86 to 89 would, therefore, be expected to occur in a certain proportion of the cases examined during the passage of urine containing haemoglobin.

TABLE 34 Examination of blood plasma for the presence of dissolved haemoglobin, during haemoglobinuria of blackwater fever. Selected from Table 33.

No. of observation	Case	Percentage of haemoglobin in plasma	Condition of urine at time of collecting plasma.
3	2	0·13 %	On centrifugalising the urine the supernatant liquid was found to be brown in colour, gave no oxyhaemoglobin bands, but yielded $\frac{1}{20}$ col. of brown precipitate on boiling after acidification with acetic acid (= about 0·2 % of haemoglobin).
16	7	0·13 %	Similar to above
19	8	0·13 %	Urine contained 0·1 % of dissolved haemoglobin. See Fig. 12
22	10	0·09 %	,, 0·03 % ,, ,, 13
25	11	0·25 %	,, 0·9 % ,, ,, 14
32	14a	0·20 %	,, 0·24 % ,, ,, 17
6	3	0·57 %	,, 1·2 % ,, ,, 10
7	,,	0·40 %	,, 1·3 % ,, ,, ,,
18	7a	0·74 %	,, 0·4 % to 1·1 % ,, ,, 11
21	10	0·85 %	,, 1·1 % ,, ,, 13
23	,,	0·30 %	,, 1·5 % ,, ,, ,,
24	,,	0·20 %	,, 0·4 % ,, ,, ,,
30	14	0·57 %	,, 1·5 % ,, ,, 16
33	15	0·56 %	,, 1·3 % ,, ,, 18
34	,,	0·42 %	,, 0·7 % ,, ,, ,,
36	17	0·65 %	,, 3·1 % ,, ,, 20
37	,,	0·67 %	,, 1·4 % ,, ,, ,,
38	,,	0·95 %	,, 1·9 % ,, ,, ,,
39	,,	0·48 %	,, 0·5 % ,, ,, ,,

Further support to this view is afforded by Table 34, in which the percentages of dissolved haemoglobin in the blood plasma in the nineteen observations last referred to are given side by side with the corresponding percentages of haemoglobin in the urine. It will be seen that, in the cases (3, 16, 19, 22, 24, 25, 32) in which the percentage of haemoglobin in the blood plasma is not increased, the percentage of haemoglobin in the urine is low, reaching to 0·9 per cent. in one case (25) and lying in the remaining six cases between 0·4 per cent. and 0·03 per cent. On the other hand, in the twelve observations in which the amount of dissolved haemoglobin in the blood plasma was increased, the percentage of haemoglobin in the urine was much higher, ranging between 0·4 per cent. and 3·1 per cent.

To sum up, then, we may say that, in the observations made in blackwater fever while the urine in the bladder still contained haemoglobin, an increased amount of dissolved haemoglobin in the blood plasma was observed in most but not in all cases.

We now turn to the second problem: is haemoglobinaemia ordinarily attended with haemoglobinuria, and, if so, what quantitative relationship between the two conditions exists? To determine this point it was necessary to produce haemoglobinaemia experimentally.

Method. Oxalated rabbit's blood was centrifugalised, the supernatant plasma pipetted off, and the red cells laked by adding a small amount of distilled water. To the solution thus obtained an amount of solid sodium chloride was added in amount sufficient to make the proportion of this salt 0·85 per cent. The red cell stromata, which were precipitated by the addition of sodium chloride, were then removed by centrifugalisation, a dark red liquid being obtained. The percentage of haemoglobin in this solution was determined by means of a haemoglobinometer reading, and a measured volume of the solution injected into a vein of the ear of the rabbit supplying the haemoglobin. At the end of three to six or more minutes, about ten drops of blood from the opposite ear were allowed to fall into 0·1 c.cm. of 1 per cent. potassium oxalate solution, the volume of the mixture being carefully measured. This was then centrifugalised, and the percentage of haemoglobin in the plasma determined usually by matching, under a comparison spectroscope, with a solution of haemoglobin of known concentration, though when the concentration of haemoglobin was high a haemoglobinometer determination was sometimes made instead. At later periods similar estimations of the haemoglobin content of the plasma were carried out. In the same way the haemoglobin content of the urine was determined, and the urine was also tested for coagulable proteid, any deposit present being submitted to microscopical examination. In Experiments 8, 9 and 10 the urine was collected continuously by means of a cannula introduced into the bladder, in the other experiments it was voided naturally.

Before experiment, the oxalated blood plasma of the rabbits employed was found to be of a very faint yellow tint, almost colourless. On spectroscopic examination no haemoglobin bands were as a rule seen, though on one occasion 0·05 per cent. of dissolved haemoglobin was met with. The weight of the rabbits ranged from 912 g. to 1,760 g., the total amount of blood the animals possessed varied from 75 c.cm. to 118 c.cm., and the ratio of blood to body weight (Table 35) was $\frac{1}{10}$ to $\frac{1}{20}$, the higher fraction being reached in the younger, and therefore lighter, animals.

The amount of haemoglobin injected was that obtainable from 0·44 g. to 7·52 g. of red cells (in the moist condition). In all except Experiments 6 and 8, Tables 35 and 37, the haemoglobin was obtained from the animal's own red cells. The amount required in the two latter experiments being too large to be conveniently taken from the animals subjected to experiment, part of the haemoglobin injected was obtained from other rabbits. The injection of laked blood produced no recognisable ill effect, the animals taking food well during the experiment and seeming in every respect normal. In Experiment 5 the animal was apparently unaffected by the injection, but died suddenly at the end of eighty-five minutes; possibly in this case death was due to embolism, for the stromata of the laked red cells were not completely removed from the haemoglobin solution before injection.

After the injection of dissolved haemoglobin into the vein of the ear, the blood plasma of the injected animal became of a reddish colour. At the end of three to twenty minutes, estimations of the amount of haemoglobin present showed that this ranged from 0·78 per cent. to 10·80 per cent. The amount present immediately after injection may be calculated in the manner indicated on p. 90, and is found to be somewhat higher, ranging between 0·81 per cent. and 12·15 per cent. As the amount of haemoglobin injected intravenously was known, the total volume of the plasma of the rabbit could be

TABLE 35. Experimental haemoglobinuria in rabbits.

No. of experiment	Amount of haemoglobin injected intravenously expressed in terms of healthy human wet red cells	Percentage of haemoglobin dissolved in blood plasma	Percentage of haemoglobin dissolved in urine	Total haemoglobin in urine	Remarks
1	1·4 g.	0·5 % at end of 60 min.	Trace in urine passed at end of 420 mins.		General condition unaffected during experiment
		0·06 % ,, 540 ,,			
2	0·81 g.	0·81 % (calculated) immediately after injection			,, ,,
		0·78 % at end of 10 mins. after injection	0·09 % ,, ,, 250 ,,	0·023 g.	
		0·49 % ,, 120 ,,	Absent ,, ,, 1320 ,,		
		0·26 % ,, 520 ,,			
		0·06 % ,, 1340 ,,			
3	3·15 g.	5·04 % (calculated) immediately after injection			,, ,,
		4·59 % at end of 11 mins. after injection.	1·70 % ,, ,, 52 ,,	0·524 g.	
		3·46 % ,, 36 ,,			
		2·12 % ,, 98 ,,	0·62 % ,, ,, 1233 ,,		
		1·81 % ,, 166 ,,			
		1·12 % ,, 218 ,,	0·35 % ,, ,, 1506 ,,		
		0·873 % ,, 279 ,,			
		0·237 % ,, 476 ,,	Absent ,, ,, 1900 ,,		
		0·145 % ,, 1346 ,,			
4	5·39 g.	6·50 % (calculated) immediately after injection			,, ,,
		5·03 % at end of 27 mins. after injection	1·32 % ,, ,, 135 ,,	1·108 g.	
		4·50 % ,, 73 ,,			
		1·76 % ,, 134 ,,	0·93 % ,, ,, 562 ,,		
		1·69 % ,, 206 ,,			
		1·65 % ,, 257 ,,	Absent ,, ,, 1582 ,,		
		1·05 % ,, 334 ,,			
		0·64 % ,, 488 ,,			
		0·14 % ,, 1386 ,,			
5	3·12 g.	4·34 % (calculated) immediately after injection			Died suddenly at the end of 85 mins.
		4·17 % at end of 21 mins. after injection	2·45 % ,, ,, 85 ,,	0·12 g.	
		4·01 % ,, 45 ,,			
		3·17 % ,, 85 ,,			
6	7·52 g.	8·59 % (calculated) immediately after injection			General condition unaffected during experiment
		8·10 % at end of 8 mins. after injection	0·633 % ,, ,, 744 ,,	0·80 g.	
		5·05 % ,, 62 ,,			
		3·26 % ,, 117 ,,			

No. of experiment	Amount of haemoglobin injected intravenously expressed in terms of healthy human wet red cells	Percentage of haemoglobin dissolved in blood plasma	Percentage of haemoglobin dissolved in urine	Total haemoglobin in urine	Remark
	2·52 g.	5·13 % (calculated) immediately after injection 4·92 % at end of 6 mins. after injection 1·85 % ,, 151 ,,	4·80 % in urine passed at end of 125 minutes	0·29 g.	General condition unaffected during experiment
7	7·00 g. injected 19½ hours later	9·20 % (calculated) immediately after injection 7·90 % at end of 33 mins. after 2nd injection 3·54 % ,, 206 ,, ,, 0·78 % ,, 544 ,, ,,	2·50 % ,, ,, 276 ,,	1·00 g.	,, ,,
	5·70 g. injected after a further period of 8½ hours. Total in 28 hours, 15·22 g.	8·50 % at end of 67 mins. after 3rd injection	4·50 % ,, ,, 79 ,, 5·00 % ,, ,, 800 ,, Absent ,, ,, 1432 ,,	1·13 g. 1·5 g.	,, ,,
8	7·33 g.	12·15 % (calculated) immediately after injection 10·80 % at end of 17 mins. after injection 6·72 % ,, 62 ,, 5·70 % ,, 88 ,, 5·30 % ,, 118 ,, 4·65 % ,, 152 ,, 3·52 % ,, 172 ,, 2·56 % ,, 315 ,, 2·00 % ,, 383 ,, 1·60 % ,, 442 ,, 1·30 % ,, 498 ,,	7·20 % ,, ,, 28 ,, 6·78 % ,, ,, 40 ,, 7·16 % ,, ,, 60 ,, 6·54 % ,, ,, 86 ,, 8·22 % ,, ,, 150 ,, 11·16 % ,, ,, 171 ,, 8·88 % ,, ,, 260 ,, 7·60 % ,, ,, 380 ,, 5·52 % ,, ,, 436 ,, 4·70 % ,, ,, 501 ,,	1·34 g.	,, ,,
9	1·3 g.	2·13 % (calculated) immediately after injection 2·10 % at end of 13 mins. after injection 1·50 % ,, 168 ,,	1·51 % ,, ,, 6 ,, 5·30 % ,, ,, 17 ,, 5·25 % ,, ,, 43 ,, 11·20 % ,, ,, 63 ,, 9·80 % ,, ,, 98 ,, 6·24 % ,, ,, 183 ,, 1·10 % ,, ,, 343 ,, Trace ,, ,, 478 ,,	0·33 g.	,, ,,
10	0·44 g.	0·96 % (calculated) immediately after injection 0·90 % at end of 5 mins. after injection 0·54 % ,, 41 ,, ,, 0·31 % ,, 79 ,, ,, 0·26 % ,, 132 ,, ,,	Nil ,, ,, 17 ,, 2·14 % ,, ,, 27 ,, 6·96 % ,, ,, 37 ,, 2·90 % ,, ,, 52 ,, 1·59 % ,, ,, 67 ,, 0·43 % ,, ,, 82 ,, Nil ,, ,, 92 ,, Nil ,, ,, 107 ,,	0·08 g.	,, ,,

calculated from the percentage present in the plasma after injection, and, as the relation between the volume of the red cells and that of the plasma had previously been determined by the aid of the haemocrit, the total volume of the blood was also by this means ascertained.* As time went on the amount of haemoglobin in the rabbit's plasma diminished at first rapidly, then more slowly, until at the end of twenty-four hours the amount present was less than 0·2 per cent.

The first specimen of the urine, after intravenous injection of dissolved haemoglobin, was, in all experiments except the first two, in which the tint was lighter, porter coloured in a layer two inches or more in thickness, and dark red or reddish brown in a layer one centimetre thick. The percentage of haemoglobin ranged from 5·0 per cent. to 0·6 per cent., reaching, however, in one experiment 0·09 per cent. In succeeding specimens of the urine the amount of haemoglobin usually became progressively smaller, and at the end of twenty-four hours disappeared. The total amount of haemoglobin eliminated in the urine after injection, ranged in the experiments made between 0·2 g. and 1·34 g. These amounts refer, however, only to unaltered haemoglobin. In addition a relatively small amount of haemoglobin was broken up in the urine, to which it imparted a brownish tint. The actual amount of haemoglobin passing into the urine would, therefore, be slightly greater than the amounts given in Column 6, Table 35. To obtain some idea of the total haemoglobin discharged into the urine, a given volume of the urine was rendered slightly acid with acetic acid, boiled, and the volume of the resulting chocolate coloured precipitate measured after centrifugalisation in a graduated tube. By comparison with a parallel series of experiments in which known amounts of red cells were laked and added to urine, the volume of the precipitate obtained after acidifying and boiling being carefully noted, the amounts of haemoglobin originally discharged into the urine were determined. The percentages obtained in this way were larger than those given by haemoglobinometer readings of the diluted urine, by an amount not exceeding 25 per cent. In these experiments the precipitate produced in the urine by boiling was indistinguishable from that obtained on boiling a solution of haemoglobin. In particular, no lighter colour, such as might be taken to indicate the presence of serum albumin or globulin could be detected, when the former precipitate was compared with the latter.

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* For further details cp. A method of estimating the total volume of blood contained in the living body, Proceedings of the Royal Society of London, 1909. Series B., vol. 81.

Table 36. Examination of oxalated blood plasma for the presence of dissolved haemoglobin, in experimentally induced haemoglobinuria in rabbits. Selected from Table 35.

No. of Experiment	Percentage of haemoglobin in plasma	Condition of urine	
1	0·5 % or less	Urine contained trace of haemoglobin	
2	0·81 % to less than 0·49 %	,, ,, 0·09 % ,,	
3	5·04 % to 3·10 %	,, ,, 1·70 % ,,	See fig. 2
4	6·50 % to 1·76 %	,, ,, 1·32 % ,,	,, 3
5	4·34 % to 3·17 %	,, ,, 2·45 % ,,	,, 4
6	8·59 % to less than 0·84 %	,, ,, 0·63 % ,,	,, 5
7	(*a*) 5·13 % to 2·20 %	,, ,, 4·80 %	
	(*b*) 9·20 % to less than 3·54 %	,, ,, 2·50 % ,,	,, 6
	(*c*) 8·50 % or more	,, ,, 4·50 %	
8	12·15 % to 10·4 %	,, ,, 7·20 % ,,	,, 7
9	2·20 % to 2·00 %	,, ,, 11·20 % (maximum)	,, 8
10	0·90 % to 0·54 %	,, ,, 7·00 % (maximum)	,, 9

In Table 36 some of the percentages of haemoglobin in blood plasma and urine are placed side by side so that the degree of haemoglobinaemia and haemoglobinuria may be compared, as was done in the case of the blackwater fever observations recorded in Table 34. The highest degree of haemoglobinaemia observed in the rabbit is 10·8 per cent., while the highest amount in blackwater fever was 0·95 per cent. It must, however, be observed that in the former case the observations were frequently repeated, and the time at which the maximum would occur was known in advance, while in the human subject the number of observations which could be made was limited and the probable course of the haemoglobinaemia was unknown, so that highest percentages were therefore in all probability not ascertained.

In comparing the degree of haemoglobinaemia with that of haemoglobinuria in experimentally induced haemoglobinaemia it must be remembered that the last three experiments in Table 36 stand apart from the others since in these the urine was collected continuously, instead of being voided at intervals as in the preceding experiments. It will be noticed that the percentage of haemoglobin in the urine voided in the first seven experiments is less than that in the blood plasma (Table 36); in Observation 8 the same is also noted, but in Observations 9 and 10 this relation is reversed in the sense that the maximum percentage of haemoglobin in the urine is considerably higher than the corresponding percentage in the blood plasma. A better comparison is, however, made if curves are constructed, the ordinates of which denote percentages of haemoglobin and the abscissae periods of time. This is done in Figs. 2 to 9.

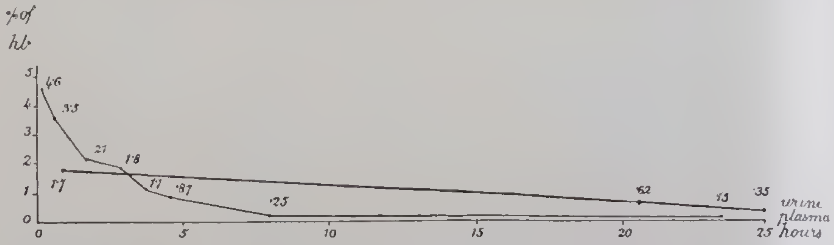

FIG. 2. Exp. 3, Table 35. Experimental haemoglobinaemia of rabbit. Percentage of dissolved haemoglobin in blood plasma and urine.

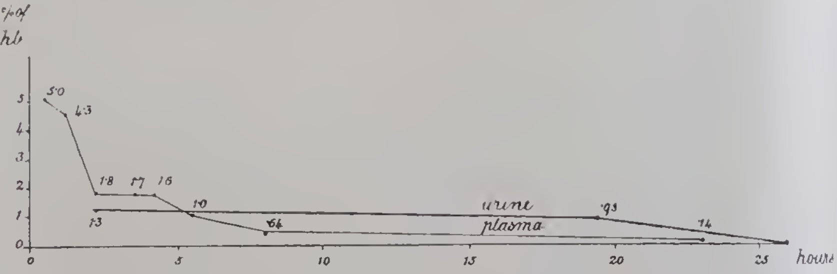

FIG. 3. Exp. 4, Table 35. Experimental haemoglobinaemia of rabbit. Percentage of dissolved haemoglobin in blood plasma and urine.

In most of these curves (Figs. 2 to 6) the percentage of haemoglobin in the plasma appears first greater, and subsequently less, than that in the urine. In Figs. 7, 8 and 9, however, it is seen that the percentage of haemoglobin in the urine in reality at the beginning of the experiment quickly rises till it considerably exceeds that in the plasma, after which it declines again with the latter. The greatest difference was observed in Experiment 9 in which the percentage of haemoglobin in the urine became at one time as much as 7 per cent., while that in the blood plasma was only 0·54 per cent

It is, of course, obvious that if in these three experiments (Figs. 7, 8, 9) the urine had been allowed to accumulate in the bladder sufficiently long no such relation would have been discoverable. As regards the earlier portions of the urine, which contained less haemoglobin than the blood plasma, in Experiments 8 to 10, in which continuous collection was practised, it is uncertain how far these represent the real state of affairs, since although the urine was

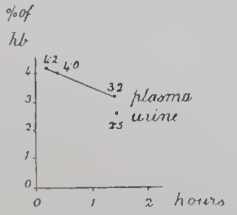

FIG. 4. Exp. 5, Table 35. Experimental haemoglobinaemia of rabbit. Percentage of dissolved haemoglobin in blood plasma and urine.

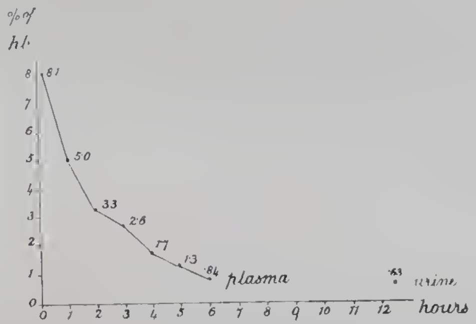

FIG. 5. Exp. 6, Table 35. Experimental haemoglobinaemia of rabbit. Percentage of dissolved haemoglobin in blood plasma and urine.

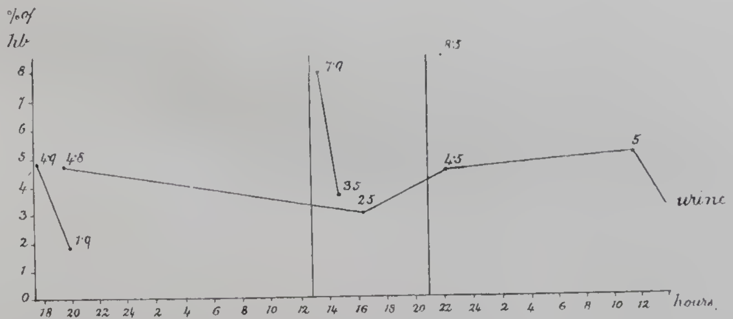

FIG. 6. Exp. 7, Table 35. Experimental haemoglobinaemia of rabbit. Percentage of dissolved haemoglobin in blood plasma and urine.

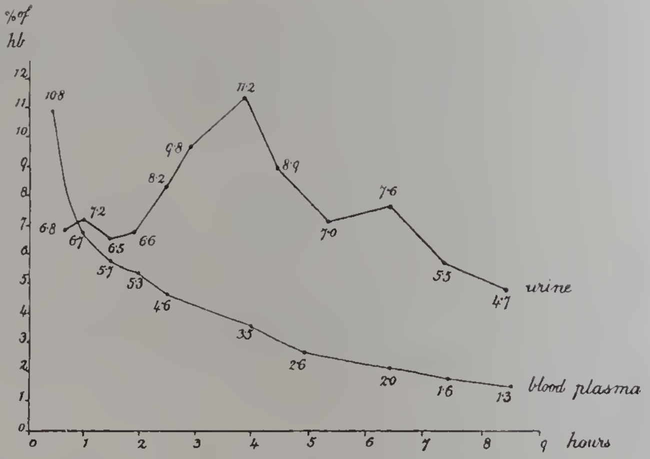

FIG. 7. Exp. 8, Table 35. Experimental haemoglobinaemia of rabbit. Percentage of dissolved haemoglobin in blood plasma and urine.

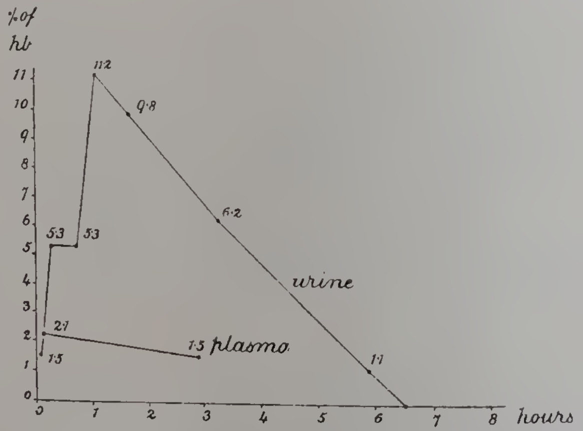

FIG. 8. Exp. 9, Table 35. Experimental haemoglobinaemia of rabbit. Percentage of dissolved haemoglobin in blood plasma and urine.

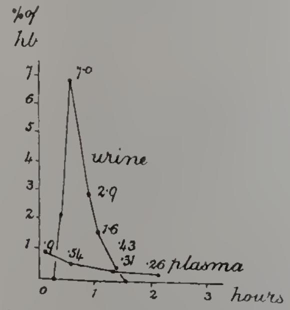

FIG. 9. Exp. 10, Table 35. Experimental haemoglobinaemia of rabbit. Percentage of dissolved haemoglobin in blood plasma and urine.

collected by means of a cannula tied in the bladder, the portion first secreted containing haemoglobin would be diluted with the normal urine still remaining in the pelves of the kidneys and ureters, and also with the very small amount yet remaining in the bladder. In the experiments recorded in Figs. 7 to 9, the urine in the glass cannula continued amber-coloured for two to five minutes after intravenous injection of dissolved haemoglobin; it then became dark red in colour, a sharp line of demarcation appearing in the cannula between the two kinds of urine. The red tint of the urine then underwent a slight increase in depth as the urine continued to be secreted. To what extent this was due to the first portions being diluted with urine still remaining in the urinary passages is not clear, though from the

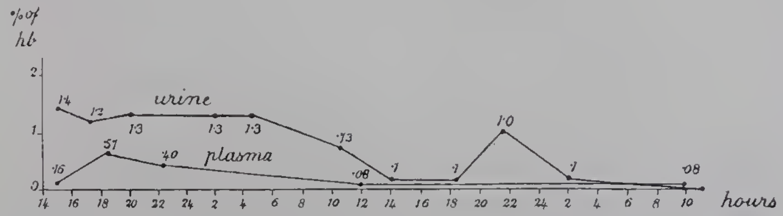

FIG. 10. Blackwater Fever, Case 3. Percentage of dissolved haemoglobin in blood plasma and urine. For further details of urine, see p. 185.

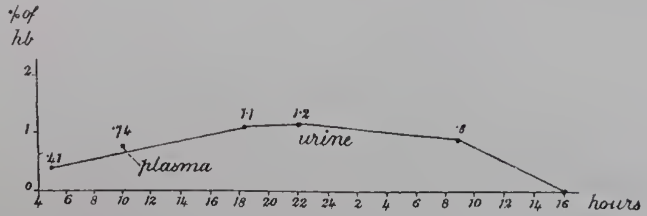

FIG. 11. Blackwater Fever, Case 7a. Percentage of dissolved haemoglobin in blood plasma and urine. For further details of urine, see p. 199.

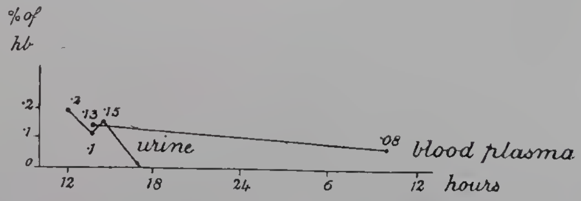

FIG. 12. Blackwater Fever, Case 8. Percentage of dissolved haemoglobin in blood plasma and urine. For further details of urine, see p. 204.

curves in Figs. 7 to 9 it can be asserted with certainty that the initial rate of elimination of urine is not the maximum rate, but continues for a time to increase.

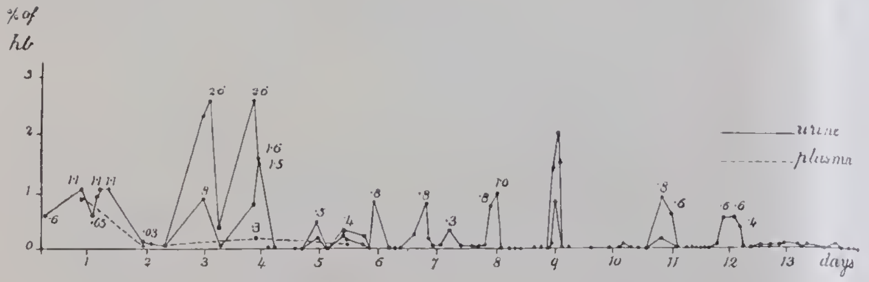

FIG. 13. Blackwater Fever, Case 10. Percentage of dissolved haemoglobin in blood plasma and urine. For further details of urine, see p. 211.

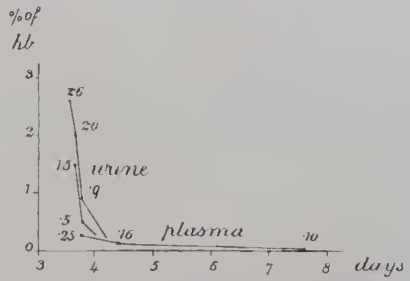

FIG. 14. Blackwater Fever, Case 11. Percentage of dissolved haemoglobin in blood plasma and urine. For further details of urine, see p. 220.

It is now possible in the light of the preceding experimental observations to study further the relation between the haemoglobinuria and the accompanying haemoglobinaemia already observed in some, but not all, of the cases of blackwater fever

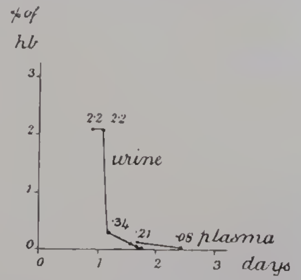

FIG. 15. Blackwater Fever, Case 12. Percentage of dissolved haemoglobin in blood plasma and urine. For further details of urine, see p. 224.

examined during the passage of urine containing haemoglobin. Turning again to Table 34 (p. 75), we find that we are here dealing with much smaller percentages of haemoglobin, especially in the case of the blood plasma, than those obtaining experimentally in rabbits. Although the observations made on blackwater fever are, in part, necessarily far less complete than those obtaining in the rabbit, and the percentages obtained cannot pretend to represent maximal

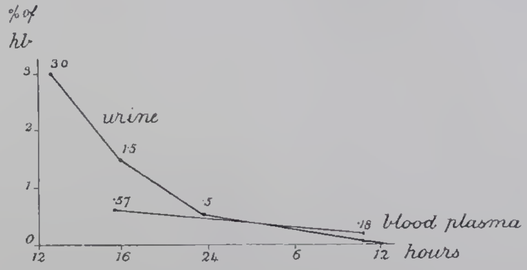

FIG. 16. Blackwater Fever, Case 14. Percentage of dissolved haemoglobin in blood plasma and urine. For further details of urine, see p. 228.

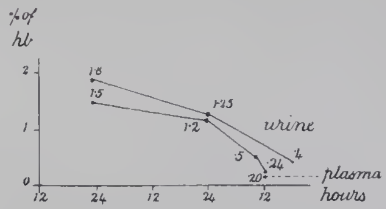

FIG. 17. Blackwater Fever, Case 14a. Percentage of dissolved haemoglobin in blood plasma and urine. For further details of urine, see p. 230.

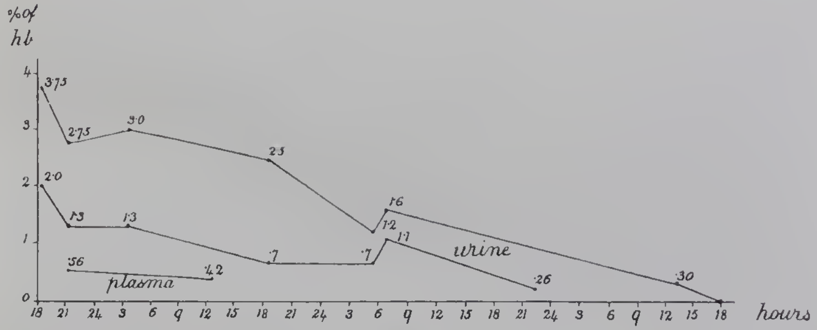

FIG. 18. Blackwater Fever, Case 15. Percentage of dissolved haemoglobin in blood plasma and urine. For further details of urine, see p. 233.

percentages, yet one feature stands out quite clearly, namely, that the percentage of dissolved haemoglobin in the blood plasma appears to be much lower than in experimentally induced haemoglobinaemia, none of the specimens of plasma obtained giving a higher percentage than 0·95. On the other hand the percentages of haemoglobin in the urine, though not quite so high as those observed under similar conditions in rabbits, are much more nearly comparable with the latter, the highest 3·1 per cent. (Observation 36, Table 34). That the degree of haemoglobinaemia observed in blackwater fever in the last thirteen experiments in Table 34 is not incompatible with the considerably higher percentages of haemoglobin met with in the urine in these cases, is shown by the experimental work in rabbits, in particular by Experiments 7 to 9, Table 36, so that there is nothing in these thirteen results (Observations 6 to 39, Table 34) to negative the supposition that the haemoglobinuria in these cases is dependent upon accompanying haemoglobinaemia. To render a comparison with the experimental results easier the series of diagrams, Figs. 10

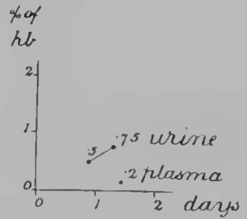

FIG. 19. Blackwater Fever, Case 16. Percentage of dissolved haemoglobin in blood plasma and urine. For further details of urine, see p. 237.

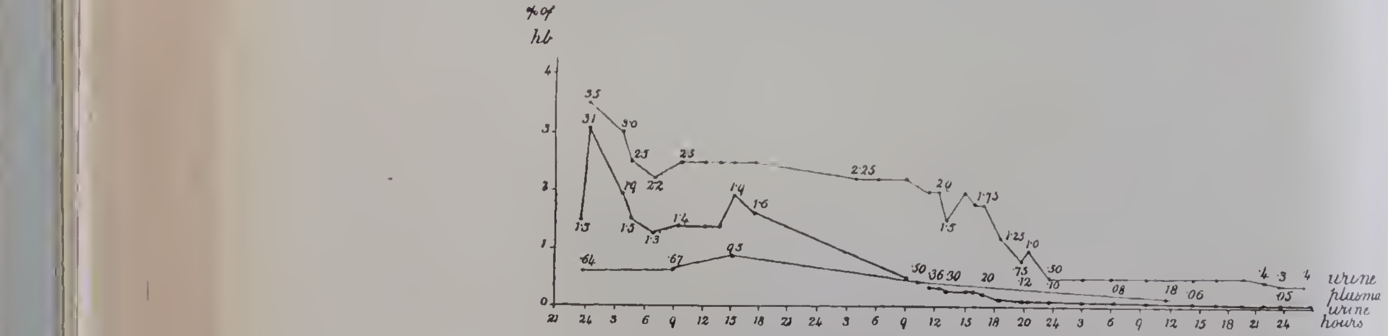

FIG. 20. Blackwater Fever, Case 17. Percentage of dissolved haemoglobin in blood plasma and urine. For further details of urine, see p. 241.

to 20, have been made. Notwithstanding the incompleteness of some of the Figures, they illustrate the wide divergence in degree between haemoglobinaemia and haemoglobinuria just referred to (Figs. 10, 13, 14, 16, 18, 20). It is observable that a fall in the percentage of dissolved haemoglobin in the blood plasma appears attended by a corresponding fall in the percentage of haemoglobin in the urine (Figs. 10, 12, 13, 16, 18, 20), just as occurs, after the initial maximum has been reached, in experimental haemoglobinaemia (Figs. 8, 9, 10). A striking point of difference between the two series of observations is that in experimental haemoglobinaemia the disappearance of haemoglobin from the blood plasma appears to be more rapid than in blackwater fever. To make this clear, a comparison in respect of time should be made between the rate of disappearance of haemoglobin in Figs. 2 to 9 (experimental haemoglobinaemia) and that occurring in Figs. 10, 12, 13, 14, 18 and 20 (blackwater fever).

If the comparison is made between the amount of haemoglobin injected intravenously and that reappearing in the urine (Table 35), it is seen that, in our experiments, the latter was only a small fraction of the former ranging between 3 per cent. in Experiment 2 and 25 per cent in Experiment 9. Thus the laked haemoglobin in the rabbit's plasma does not pass through the kidneys except in a relatively small amount, the greater part of the dissolved haemoglobin being dealt with in the body. We have here a striking parallel with the effect of injecting egg albumin into animals. In the latter case the foreign proteid injected is, in carnivora, broken up in the body, the nitrogen it contains being eliminated as urea in the urine, and only a small fraction escaping through the kidneys unchanged.*

In the experiments recorded in Table 35 repeated examinations of the blood plasma were made so that it becomes possible to study the rate at which elimination of dissolved haemoglobin has taken place. This proceeded rapidly at first and subsequently progressed more slowly. In one Experiment (4, Table 35) a marked irregularity in the rate of disappearance occurred, the percentage of dissolved haemoglobin in the plasma remaining almost unchanged for two hours. The reaction rate formula for bimolecular and multimolecular reactions does not apply to these experiments. The rate of disappearance of haemoglobin follows, however, fairly closely a monomolecular course, as the values of K, calculated in the fourth column of Table 37, show. This will be further evident when the percentages calculated in the last column of this table are compared with those determined by experiment, in the third column. It thus becomes possible to calculate the haemoglobinaemia present immediately after injection, and from this the total volume of blood in the animals at the beginning of the experiment can be determined (Col. 3, Table 35). When the dissolved haemoglobin has been reduced to less than 10 per cent. of its original amount, further destruction proceeds with considerable and rapidly increasing slowness. The rate of disappearance thus is similar to that observed in Table 17. Contrary to what would be expected there seems to be considerable difficulty in removing the last traces of haemoglobin from the plasma. It may here be observed that rabbits which have been injected with dissolved haemoglobin show small percentages of haemoglobin in their blood plasma for many days afterwards, in this respect resembling human beings and differing from normal rabbits. The curious change in the rate of disappearance of haemoglobin, exhibited in Experiment 4, in which the percentage of haemoglobin remained almost unaltered between the hundred and thirty-fourth and the two hundred and fifty-seventh minutes, is difficult to interpret. The rate of disappearance of haemoglobin is relatively slow in Experiments 2, 5 and 9, in which the value of K is 0·00105 ; in the other experiments K ranges between 0·0029 and 0·0056. These values stand, except in Experiment 9, in a definite relation to the age of the animal used for experiment ; in Experiments 2, 5 and 9 the animals employed were young, and the total blood was, as already mentioned, about one-tenth of the body weight.

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* Friedemann and Isaac. Zeitschr. f. exper. Pathologie, 1905, Bd. 1, S. 513, quoted by v. Noorden, Pathology of Metabolism, London, 1907, Vol. 2, p. 91.

The question arises, does the disappearance of the dissolved haemoglobin depend upon decomposition occurring in the blood plasma ? This enquiry cannot be directly settled by experiment in vitro with the non-oxalated plasma of the rabbit, owing to the rapidity with which clotting takes place. In Table 38 are recorded some observations made with oxalated plasma, and with plasma defibrinated by shaking with glass beads. In Experiment 1 the

TABLE 37. The rate of disappearance of dissolved haemoglobin from the blood plasma in the living body of the rabbit. Cp. Table 35.

No. of Experiment (Table 29)	t	Corresponding relative percentage of haemoglobin (found by experiment)	$\frac{1}{t_x - t_0} \log \frac{C_0}{C_x}$ *	Calculated relative percentage of haemoglobin
2	0 min.	[100]		[100] [K = 0·00105]
Weight of rabbit = 1083 g.	10 ,,	96		96
	170 ,,	59	0·00116 Mean	68
$\frac{\text{Blood}}{\text{Body weight}} = \frac{1}{10}$	520 ,,	33	0·00095 0·00105	27
	1320 ,,	8	0·00084	4
3	0 ,,	[100]		[100] [K = 0·0030]
	11 ,,	91	0·0031	92
	36 ,,	69	0·0045	78
Weight of rabbit = 1440 g.	98 ,,	42	0·0038	51
	166 ,,	36	0·0027 Mean	32
$\frac{\text{Blood}}{\text{Body weight}} = \frac{1}{14·5}$	218 ,,	22	0·0030 0·0030	22
	279 ,,	17	0·0026	15
	476 ,,	5	0·0028	4
	1346 ,,	3	0·0011	0·0009
4	0 ,,	[100]		100 [K = 0·0029]
	27 ,,	78	0·0041	81
	73 ,,	69	0·0022	62
Weight of rabbit = 1710 g.	134 ,,	27	0·0042 Mean	43
	206 ,,	26	0·0028 = 0·0029	26
$\frac{\text{Blood}}{\text{Body weight}} = \frac{1}{15}$	257 ,,	25	0·0023	18
	334 ,,	16	0·0024	11
	448 ,,	10	0·0021	4
	1386 ,,	2	0·0012	0·01
5	0 ,,	[100]		100
Weight of rabbit = 1025 g.	21 ,,	96	0·00081 Mean	95 [K = 0·00105]
$\frac{\text{Blood}}{\text{Body weight}} = \frac{1}{11}$	45 ,,	92	0·00075 0·00105	90
	85 ,,	73	0·00160	81
6	0 ,,	[100]		100
	8 ,,	94	0·0031	94 [K = 0·00305]
Weight of rabbit = 1710 g.	62 ,,	59	0·0037	64
	117 ,,	38	0·0036	44
$\frac{\text{Blood}}{\text{Body weight}} = \frac{1}{15}$	180 ,,	31	0·0030 Mean	28
	237 ,,	19	0·0024 0·00305	19
	297 ,,	15	0·0028	12
	358 ,,	10	0·0028	8
7	0 ,,	[100]		
	6 ,,	96	0·0029	
Weight of rabbit = 912 g.	151 ,,	36		
	0 ,,	[100]		100 [K = 0·0020]
$\frac{\text{Blood}}{\text{Body weight}} = \frac{1}{12}$	33 ,,	86		86
	206 ,,	38	0·0020 Mean	39
	544 ,,	8	0·0020 0·0020	8
	67 ,,	—	—	
8	0 ,,	[100]		[100]
	17 ,,	89	0·0058	89 [K = 0·0030]
	62 ,,	55	0·0039	58
Weight of rabbit = 1210 g.	88 ,,	47	0·0032	54
	118 ,,	44	0·0027 Mean	44
	152 ,,	38	0·0031 0·0030	35
$\frac{\text{Blood}}{\text{Body weight}} = \frac{1}{15}$	172 ,,	29	0·0021	30
	315 ,,	21	0·0020	11
	383 ,,	16	0·0020	7
	442 ,,	13	0·0019	5
	498 ,,	11		1
9	0 ,,	[100]		
Weight of rabbit = 1710 g.	13 ,,	99		
$\frac{\text{Blood}}{\text{Body weight}} = \frac{1}{18}$	168 ,,	70	0·0009	
10	0 ,,	[100]		100 [K = 0·0056]
Weight of rabbit = 1760 g.	5 ,,	94		94
	41 ,,	56	0·0062 Mean	56
$\frac{\text{Blood}}{\text{Body weight}} = \frac{1}{20}$	79 ,,	32	0·0062 = 0·0056	36
	132 ,,	27	0·0042	18

* In Experiments 2, 7, 9 and 10 the formula $\frac{1}{t_x - t_1} \log \frac{C_1}{C_x}$ is employed

haemoglobin and plasma employed were those of Rabbit 5, Tables 35 and 37; in Experiment 3, those of Rabbit 6, Tables 35 and 37. In all three experiments the disappearance of laked haemoglobin took place very slowly, compared with that occurring in the living body, less than 30 per cent. being broken up in six and a half hours. No data are available to indicate the rate at which in the circulating blood, destruction is effected solely by the plasma, nor do our experiments permit any conclusion to be drawn as to the part played by the body tissues in the removal of haemoglobin. The disappearance of the dissolved haemoglobin is also, to a small extent, affected by the action of the kidneys. To what extent this affects the percentage present in the blood plasma cannot be precisely determined, since it is unknown how far the amount of water leaving the blood to pass through the kidneys is replaced by water absorbed from the contents of the stomach and intestines.

SUMMARY

The principal points in the foregoing may be summed up as follows:—

1. In oxalated blood plasma obtained from healthy individuals, 0·10 per cent., or less, to as much as 0·25 per cent. of dissolved haemoglobin was found; in oxalated blood plasma obtained during blackwater fever, while the urine in the bladder still contained haemoglobin, the amount of dissolved haemoglobin was usually, but not always, greater than this, ranging between 0·30 per cent. and 0·95 per cent.

2. Haemoglobinaemia produced experimentally in the rabbit is accompanied by haemoglobinuria, the percentage of haemoglobin in the urine quickly surpassing that in the blood plasma (Experiments 8-10, Table 35), and subsequently falling as the latter diminished.

3. The disappearance of dissolved haemoglobin from the blood plasma proceeds in the living body of the rabbit at approximately the same rate as a monomolecular chemical reaction.

TABLE 38. Decomposition of dissolved haemoglobin in oxalated or defibrinated plasma. Temperature of experiment 37° C

No. of Experiment	Source of plasma and haemoglobin	Percentage of plasma	Percentage of 0·9 % NaCl	Percentage of laked haemoglobin		Haemoglobin remaining at end of experiment	Remarks
1	Rabbit	80 % (oxalated)	13·44 %	6·56 % at beginning of experiment	4·64 % at end of 395 minutes	70·7 %	Solution of laked haemoglobin added to plasma
2	Goat	Undiluted (defibrinated)	Nil	2·88 % at beginning of experiment	2·36 % at end of 480 minutes	82·0 %	Haemoglobin dissolved during defibrination
3	Rabbit	Undiluted (defibrinated)	Nil	4·80 % at beginning of experiment	4·14 % at end of 300 minutes	86·2 %	Haemoglobin dissolved during defibrination

IV. MECHANISM OF PRODUCTION OF SUPPRESSION OF URINE IN BLACKWATER FEVER.

With a view of throwing light upon the mechanism of production of blackwater fever, the action of quinine on red cells, the relation between haemolysinaemia and blackwater, and that existing between haemoglobinaemia and haemoglobinuria, have been studied in the preceding sections. Before proceeding to consider (in the next section) how far the data obtained in the last section are capable of accounting for the haemoglobinuria of blackwater fever, it will be convenient to deal, first of all, with a not uncommon sequel of blackwater, namely, suppression of urine, since it may readily be surmised that the mechanism of production of this condition is closely linked up with that of the haemoglobinuria of blackwater fever. The investigation of the mechanism of suppression of urine in blackwater fever is in its turn intimately associated with the study of another phenomenon not hitherto referred to, that is the occurrence of casts in the urine. In the cases of blackwater fever which came under our observation, these were met with not only during the period of haemoglobinuria and for a short time after its cessation, but also during suppression, when only a very small amount of amber-coloured urine was being passed. Casts were also present during the haemoglobinuria experimentally produced in rabbits, already described in the preceding section. For convenience of description, it will be better to take first the appearance of the suspended matter in the urine in experimental haemoglobinuria; then that of the suspended matter in the urine in blackwater fever; after which the condition of the kidneys in the latter condition will be described, and lastly the bearing of the data thus obtained upon the mechanism of production of suppression of urine will be considered.

The urine of all the rabbits in which haemoglobinuria had been produced experimentally (Table 35) contained, with one exception, solid matter at the time the urine was voided. The urine was at this time free from the ordinary crystalline or amorphous deposit found in urine, especially upon standing, and the solid matter which it contained (cp. Table 39) consisted of casts containing granules of a more or less dark colour, scattered sometimes in very small numbers and irregularly (Figs. 22, 24), sometimes very densely packed together (Figs. 23, 24), the casts then becoming of a dark brown or reddish brown, almost black, colour. The size of the granules varied from 0·5 μ, or less, to as much as 4 μ to 4·5 μ, not infrequently both fine and coarse granules being present together (Figs. 22 and 24). Free granules were also met with, sometimes, if not generally, obviously due to the breaking up of the casts. The free granules appeared in the form of masses, held together by colourless material

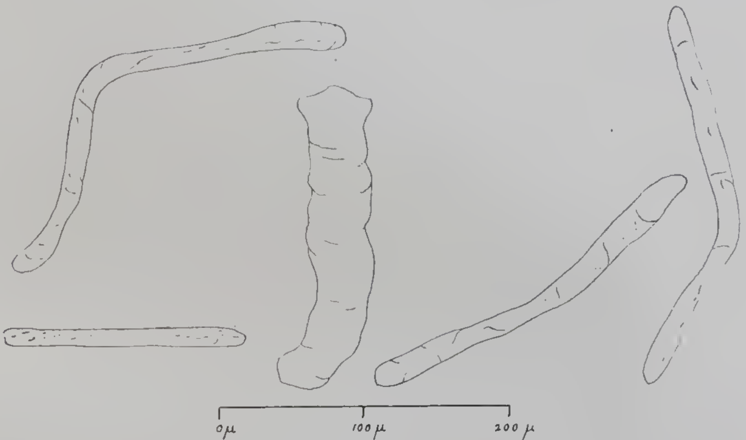

FIG. 21. Hyaline casts in urine of rabbit (Exp. 2, Table 35) four hours after intravenous injection of dissolved haemoglobin. Magnification 180 diameters.

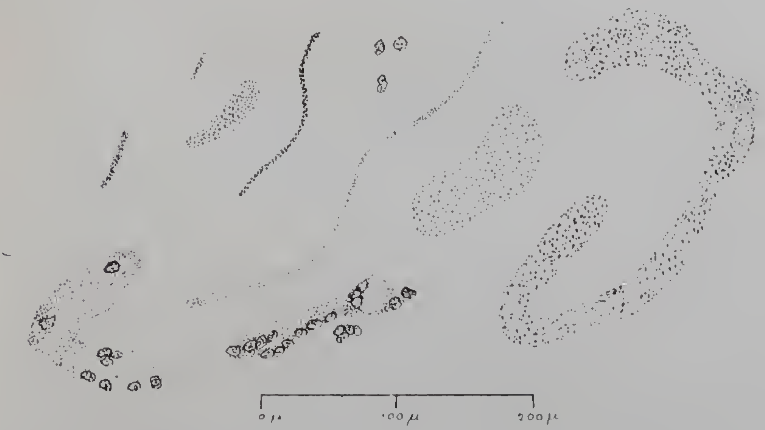

FIG. 22. Granular and epithelial casts in urine or rabbit (Exp. 3, Table 35) twenty hours after intravenous injection of dissolved haemoglobin. Magnification 180 diameters.

of a very soft consistence; sometimes they appeared to be quite unconnected with one another. The granules occasionally on standing became partly obliterated as the result of decomposition of the urine. When this occurred, as also when the granules were scanty, it became possible to observe the character of the matrix of the casts. This was of a hyaline material, not unfrequently showing creases or bends (Figs. 21, 23). Sometimes the casts exhibited here and there small masses suggestive of nuclei. In the unstained condition it was difficult to decide whether these were really nuclei or

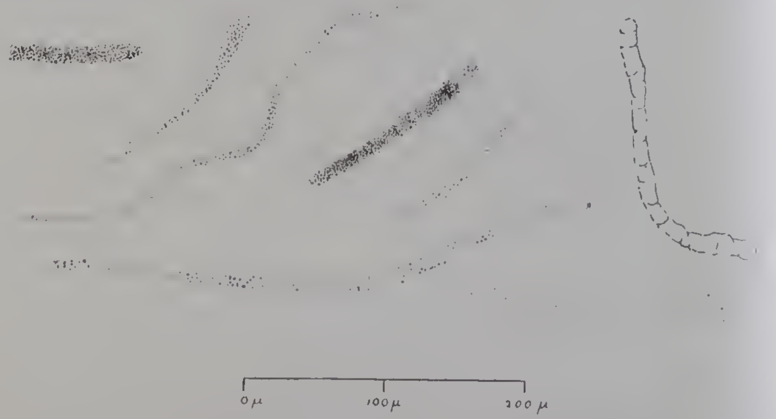

FIG. 23. Granular and hyaline casts in urine of rabbit (Exp. 5, Table 35) eighty-five minutes after intravenous injection of dissolved haemoglobin. Magnification 180 diameters.

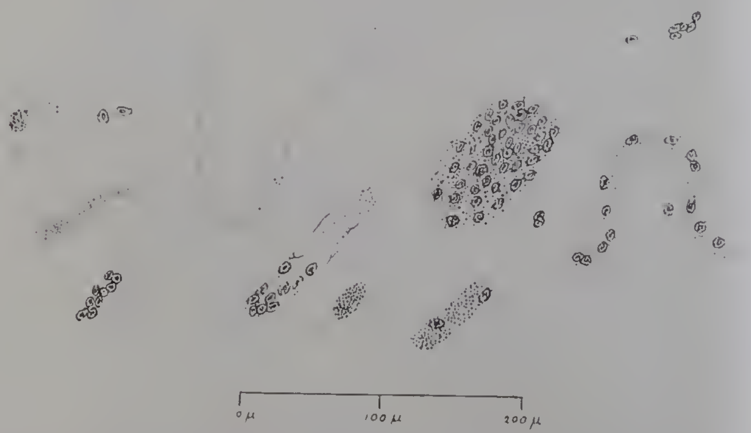

FIG. 24. Granular and epithelial casts in urine of rabbit (Exp. 6, Table 35) twelve and a half hours after intravenous injection of dissolved haemoglobin. Magnification 180 diameters.

not, but when a little methylene blue solution was added these masses could be recognised to be nuclei resembling those of the epithelial cells of the renal tubule, from which they obviously arose. The casts varied considerably in length, being from 20μ to as much as 400 μ long, their diameter ranging from 3 μ to 30 μ, or more (Figs. 21 to 24). The size of the degenerated nuclei ranged from 7 μ to 12 μ. The nuclei were usually very few in number, but occasionally became numerous, the casts then becoming epithelial casts (Figs 22, 24). Free epithelial cells with degenerated nuclei were also found, sometimes singly, sometimes in groups of two and three, in the latter case forming a transitional condition between single free cells and epithelial casts. All the free cells met with in the haemoglobinous urine of Experiments 1 to 10, Tables 35 to 39, were of spheroidal form, and appeared to be derived from the uriniferous tubules. The urine, when examined before the injection of haemoglobin, was always free from cells. The amount of solid matter contained in the haemoglobinous urine in these experiments was very variable (Table 39), being sometimes very small, sometimes abundant, forming on standing a precipitate of as much as a sixth of a column. This variation appeared in Experiments 9 and 10 (Figs. 8 and 9) to be

TABLE 39. Characters of deposit from urine of rabbit during haemoglobinuria experimentally induced. Cp. Tables 35 and 37.

No of Experiment	Colour	Amount	Microscopic characters
1	Brown	Very small	No casts
2	Brown	Very small	Hyaline casts with here and there fine granules (Fig. 21)
3	Greyish black	Very small	Granular casts (large and fine granules), very few renal epithelial cells (Fig. 22)
4	Chocolate coloured	Abundant ($\frac{1}{20}$ col.)	Granular casts (large and fine granules) and granular debris
5	—	Very small	Granular casts (large and fine granules) and hyaline casts (Fig. 23)
6	Chocolate coloured	Abundant ($\frac{1}{30}$ col.)	Granular casts (coarse and fine granules), free granules, renal epithelial casts (Fig. 24)
7	Chocolate coloured	Small	Granular casts and free granules
8	—	Small	Granular and renal epithelial casts
9	Chocolate coloured	Abundant ($\frac{1}{8}$ col.)	Granular casts and renal epithelial cells
10	Chocolate coloured	Abundant ($\frac{1}{6}$ col.)	Granular casts and renal epithelial cells

related to the percentage of haemoglobin in the urine, being most abundant when the latter was highest, but further experiments are required to settle this point definitely. The deposit from the urine on centrifugalisation was always dark in colour, sometimes of a dark chocolate colour, sometimes of a greenish black colour. Red cells were not met with. The deposit diminished in amount as the haemoglobinuria lessened, but was still present for about a day after the haemoglobinuria had ended.

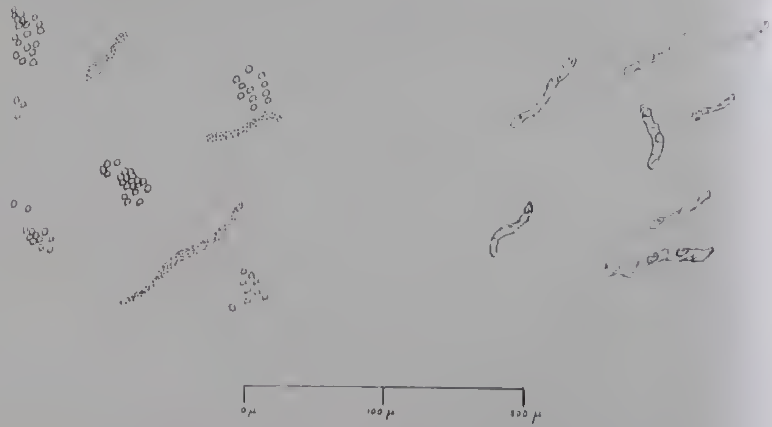

FIG. 25. To left, deposit from chocolate-coloured urine of Case 2 (second day), consisting (in addition to pus cells and bacteria, not sketched) of red cells, more or less decolourised, together with a few granular casts. To right, deposit from dark amber-coloured urine of Case 5 (fifth day), consisting of hyaline casts, in which a few indistinct nuclei can be seen. Magnification 180 diameters.

Similar casts and free epithelial cells were also found in the chocolate coloured deposit of the urine during redwater in cattle and dogs, due to piroplasmosis.

The urine in the twenty attacks of blackwater fever studied, contained, in all cases in which this point was determined, that is in all except Cases 1 and 12, Table 40, solid matter chiefly made up of granular casts and granular débris, similar in appearance to that already described in experimental haemoglobinuria in rabbits When unmixed with the crystalline and amorphous deposits formed in urine which has stood for some time, the suspended matter was found on centrifugalisation to be of a chocolate-brown colour. As in experimental haemoglobinuria, it varied considerably in amount in different cases, being sometimes scanty, sometimes when allowed to sediment amounting to a fifth of a column of the urine. As in the former experiments, so here also it is not possible, from the data at present available, to state the conditions which determine the amount of suspended matter; no clear relationship between the amount of suspended matter, on the one hand, and the percentage or the total amount of haemoglobin eliminated in the urine, on the other, is recognisable by a comparison of Table 40 with Tables 43 and 45.

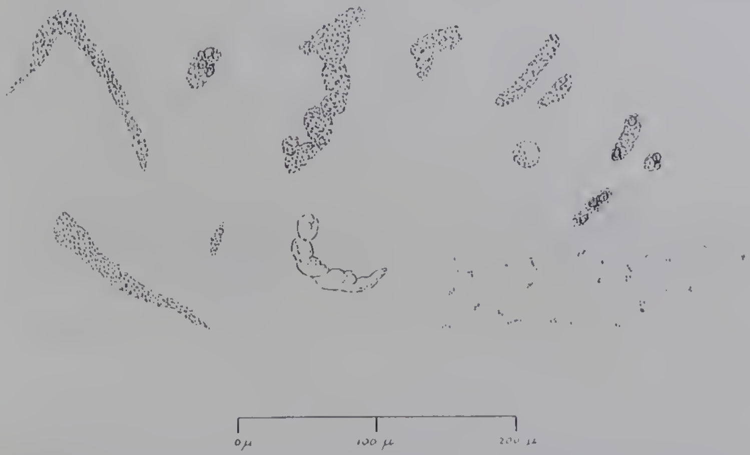

Fig. 26. Deposit from porter-coloured urine of Case 3 (second day). Granular casts and free granules are seen. Also one hyaline cast. Nuclei can be recognised in four of the casts. Magnification 180 diameters.

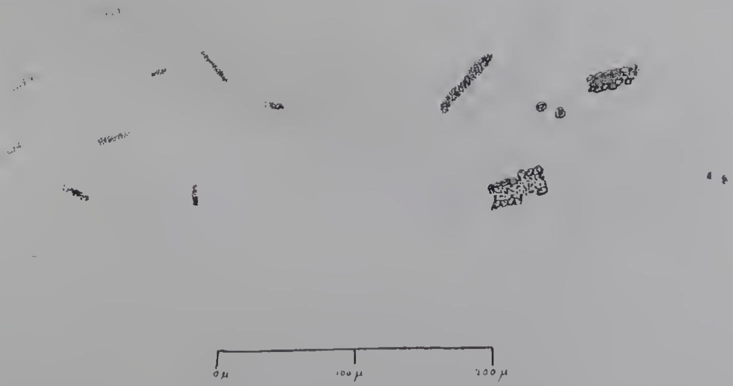

Fig. 27. To left, deposit from amber coloured urine of Case 4 (third day), consisting of small granular casts. To right, deposit from port wine-coloured urine of Case 8 (third day), showing three granular casts, two of which contain degenerated nuclei, derived from a renal tubule; two squamous cells are also seen. Magnification 180 diameters.

The amount of deposit diminishes with the haemoglobinuria, but after the urine has remained free from haemoglobin as long as three days, casts may still be found in it.

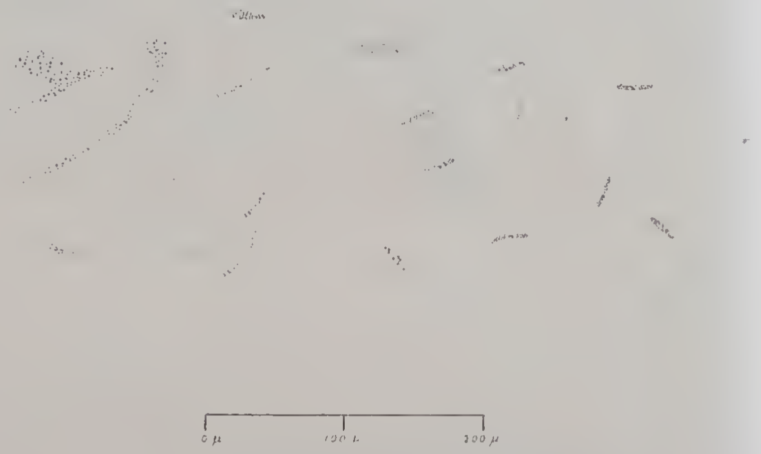

Fig. 28. Deposit from porter-coloured urine of Case 7 (second day), consisting of granular casts, the granules being dense in the smaller casts. Magnification 180 diameters.

The appearance of the casts and granules in the urinary deposit in the blackwater fever cases examined (Figs. 25 to 31) presented slight points of difference from that seen in the rabbit's urine. The length of the casts varied from 15 μ to 200 μ, or more, their diameter

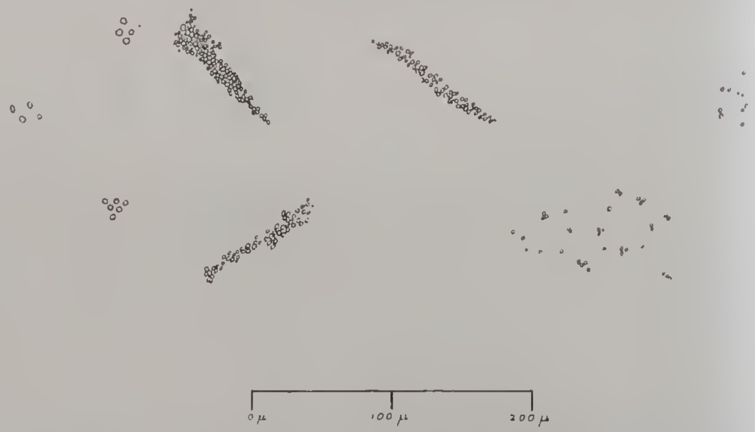

Fig. 29. Deposit from porter-coloured urine of Case 7a (first day), consisting of granular casts and débris, and (to left) a few red cells. The granules are of dark brown colour and large size, having a diameter of 4μ to 4·5μ. Magnification 180 diameters.

ranging between 3 μ and 25 μ, rarely more. The casts were brown in colour, the depth of colour depending upon the sparsity or abundance of the granules present. When the casts contained very few granules their hyaline matrix became visible (Figs. 25, 26). Sometimes the granules were scattered irregularly and in small numbers (Figs. 25, 28, 31), sometimes very densely throughout the casts (Fig. 30). The granules were sometimes very fine, not more than 0·5 μ in diameter (Figs. 25, 28), sometimes very coarse, reaching 4 μ in diameter (Fig. 29); intermediate forms were common (Figs. 26, 30).

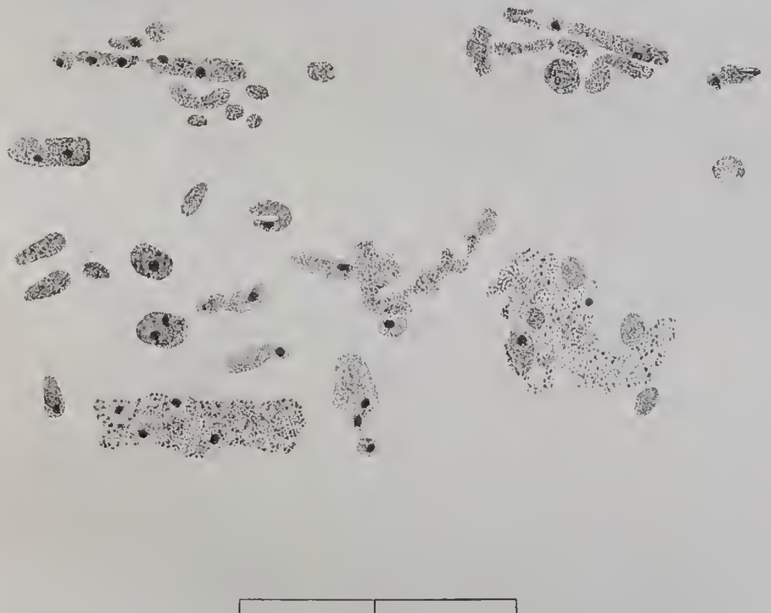

Fig. 30. Deposit from porter-coloured urine of Case 15 (third day), stained with a watery solution of methylenblue. The deposit consists of granular casts and masses, in some of which nuclei of renal origin are seen. The granules are mostly fine, but some coarse granules are seen. A few free renal cells are also present. Magnification 180 diameters.

The colour was brown, the largest granules being the darkest; but occasionally variation in the depth of colour was met with irrespective of size. Masses of granules were also met with, some obviously representing broken-down casts, as well as free granules (Fig. 29), as in haemoglobinuria of the rabbit. The consistence of the granular casts varied considerably, some being hard and dry (Figs. 27, 28, 30), others being soft and presenting a swollen appearance (Figs. 26, 3 the former were sometimes small, the latter of large size, as the sketches indicate. Hyaline casts (Figs. 25, 26) were much less frequent than granular casts. Casts containing one or more nuclei (Figs. 25, 30, 31), and epithelial casts (Figs. 27, 30, 31), were not uncommon. Free cells and cell masses were also seen (Figs. 27, 30, 31); the cells were mononuclear, of spheroidal aspect, with sometimes moderate, sometimes abundant, cytoplasm, and obviously came from the uriniferous tubules. Red blood cells, which were not met with in the rabbit's urine during experimental haemoglobinuria, were

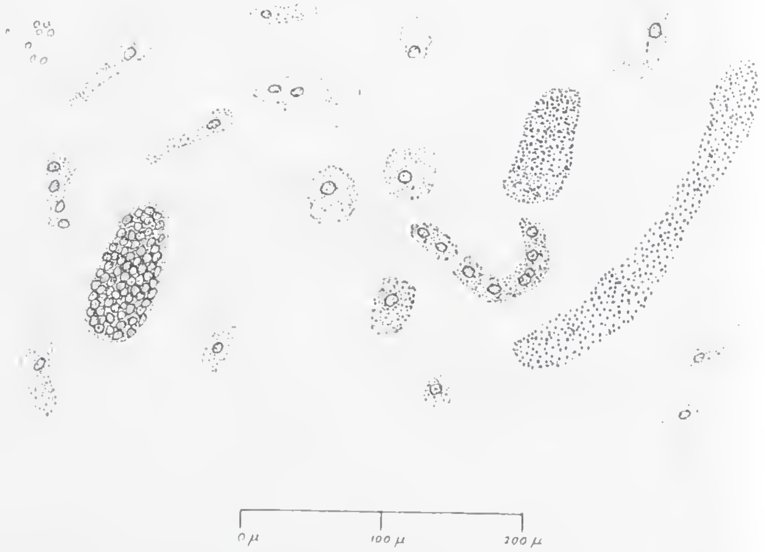

FIG. 31. Deposit from porter-coloured urine of Case 17 (second day), consisting of numerous epithelial cells; also in smaller numbers granular and epithelial casts and a few red blood cells, the latter being shown in the upper left hand corner of the sketch. Magnification 180 diameters.

encountered during the haemoglobinuria of blackwater fever in ten out of the twenty attacks investigated (Tables 40 and 43; Figs. 25, 29, 31). These were usually present in very small numbers, but in Case 2 (pp. 178 to 181) their number was considerable; this case will be referred to at length later (p. 149). In three cases (7a, 10, 11; cp. clinical notes, pp. 195, 207 and 217) red cells were not present at first, but as the haemoglobinuria was passing off made their appearance. All the above formed elements disappeared completely within three or four days after the disappearance of haemoglobinuria, except in Cases 7a and 11, in which suppression of urine occurred; in these casts or plugs continued for eight and five days respectively, until death occurred.

Occasionally squamous epithelial cells were met with, and sometimes also pus cells (Cases 2 and 9), the latter persisting after complete recovery from blackwater fever. Short bacilli were present in one case (Case 2), both before and after the attack. In no other case were bacteria seen, and in no case were organisms or cells other than those already described met with.

TABLE 40. Characters of deposits from urine during haemoglobinuria of blackwater fever.

Case	Colour	Amount	Microscopic characters
1	—	—	Red cells in very small numbers
2	Chocolate coloured	Abundant ($\frac{1}{4}$ col.)	Numerous red blood cells, pus cells, granular casts, free granules, epithelial cells (Fig. 25)
3	Whitish	Abundant ($\frac{1}{6}$ col.)	Granular casts and masses (fine granules), epithelial casts (on seventh day very few red cells) (Fig. 26)
4	—	Small	Granular casts and masses (Fig. 27
5	Brownish	Small	Hyaline masses or casts (Fig. 25)
6	—	Small	Granular casts, renal epithelial cells, few red cells
6a	—	Small	Red cells, granular casts and masses, renal epithelial cells
7	Dark brown	Abundant ($\frac{1}{8}$ col.)	Granular casts (fine granules) and masses, renal epithelial cells (Fig. 28)
7a	Dark brown	Abundant ($\frac{1}{15}$ col.)	Granular casts (coarse granules), renal epithelial cells, and red cells (Fig. 29)
8	—	Small	Granular casts, few renal epithelial and red cells (Fig. 27)
9	Whitish	Small ($\frac{1}{10}$ col.)	Pus cells, granular masses and casts (examined on eighth day)
10	—	Abundant ($\frac{1}{10}$ col.)	Granular casts and masses, renal epithelial cells (on fourth day very few red cells)
11	—	Abundant	Granular casts and masses, renal epithelial cells (on fourth day very few red cells)
12	—	Abundant	Granular and hyaline casts, renal epithelial cells
13	—	—	—
14	—	Small	Granular and epithelial casts and renal epithelial cells
14a	Brownish white	Small ($\frac{1}{40}$ col.)	Granular casts (coarse granules) epithelial casts
15	Brown	Small ($\frac{1}{60}$ col.)	Granular, hyaline and epithelial casts, free granules (coarse and fine) (Fig. 30)
16	Whitish	Small	Granular casts, renal epithelial cells
17	Chocolate coloured	Abundant ($\frac{1}{12}$ col.)	Granular and epithelial casts (coarse and fine granules) renal epithelial cells, few red cells (Fig. 31)

During suppression of urine in blackwater fever (Cases 7a and 11, Table 41) casts continued to be found in the urine. The amount of deposit obtainable on standing or centrifugalisation was extremely small. In Case 7a it appeared of a brownish colour and in Case 11 of a whitish aspect, but its colour was difficult to estimate owing to the small amount obtainable; in the former case, for example, if a sufficient amount of deposit had been available, it would probably, judging from its microscopical characters, have been of a dark reddish brown or chocolate colour. The deposit consisted principally of dark granular casts, which, in Case 7a, were just visible to the naked eye, being about 100 μ to 150 μ long and 40 μ to 60 μ broad.

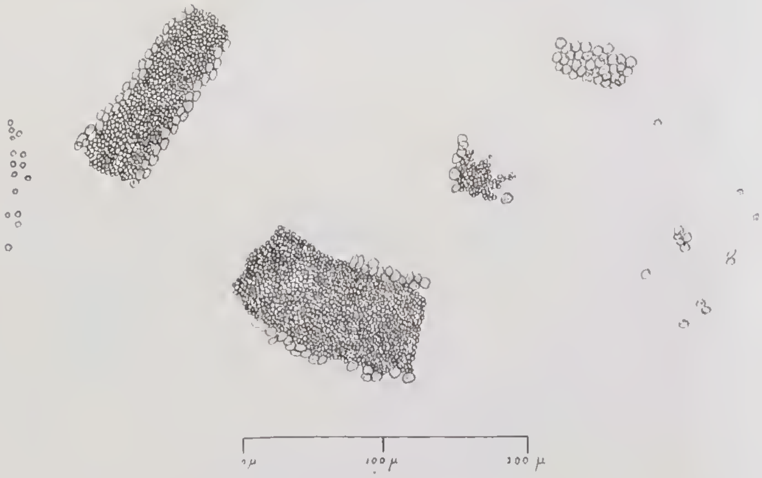

FIG. 32. Deposit from light yellow-coloured urine of Case 7a (third day, during suppression of urine). On the left are a few red blood cells. In the middle are two large casts and a fragment of a third, containing large coarse granules, closely packed and bounded externally by epithelium derived from the collecting tubules. To the right are renal epithelial cells and cell masses. Cp. with Figs. 28 and 29. Magnification 180 diameters.

The granules were of more or less spherical form, very coarse, having a diameter of about 5 μ, of a dark reddish brown colour, in their aggregated condition somewhat resembling blood clot, but of a deeper colour, and were densely packed together, so that it was sometimes difficult to discern the outlines of the individual granules (Fig. 32). No fine or medium sized granules were observed. The casts were covered externally with a layer of epithelium, and from their size and the appearance of the epithelium it is clear that most of these casts were formed in the ducts of Bertini which they completely plugged. In addition, masses of epithelial cells and single epithelial cells, both presumably of renal origin and apparently separated off from the casts, were met with, as also red cells in small numbers in both Case 7a (Fig. 32) and Case 11. Casts and red blood cells continued in the urine during the whole period intervening between cessation of haemoglobinuria and death. No bacteria or other formed elements were met with; hyaline casts were absent. The amount of the deposit, which could not be accurately measured, owing to the small amount of urine passed, but was relatively as well as actually extremely small, did not appear to vary much from day to day during the period of suppression, nor did the composition of the deposit show any marked change during this period.

TABLE 41. Characters of deposit from urine during suppression in blackwater fever (Cases 7a and 11). Case 16 is also given in this Table.

Case	Colour	Amount		Microscopical characters	Coagulable proteid
7a—2nd day	Brownish	[See p. 199]	very slight	Red cells, granular casts (coarse granules) surrounded by renal tubule cells, granular débris, free renal cells. [Fig. 32]	Precipitate $\frac{1}{4}$ col. to $\frac{2}{3}$ col., of slightly brownish white colour
,, 3rd ,,	,,	[Urine 21 c.c.]	,,		
,, 4th ,,	,,	[Urine 11 c.c.]	,,		
,, 5th ,,	,,	[Urine 17 c.c.]	,,		
,, 6th ,,	,,	[Urine 33 c.c.]	,,		
,, 7th ,,	,,	[Urine 48 c.c.]	,,		
,, 8th ,,	,,	[Urine 35 c.c.]	,,		
,, 9th ,,	,,	[Urine 23 c.c.]	,,		
,, 10th ,,	,,	[Urine 43 c.c.]	,,		
11—4th ,,	Nearly white	[Urine 48 c.c.]	slight	Granular casts, epithelial cells, very few red cells	Precipitate $\frac{2}{5}$ col. to $\frac{1}{2}$ col. of white colour
,, 5th ,,	,,	[Urine 92 c.c.]	,,		
,, 6th ,,	,,	[Urine 73 c.c.]	,,		
,, 7th ,,	,,	[Urine 93 c.c.]	,,		
,, 8th ,,	,,	[Urine 85 c.c.]	,,		
,, 9th ,,	,,	[Urine 8 c.c.]	,,		
16—4th ,,	—	[Urine 336 c.c.]	very slight	[On 2nd and 3rd days, deposit small, consisting of granular casts and epithelial cells]	$\frac{1}{10}$ col. white precipitate [sp. gr. 1·015]
,, 5th ,,	—	[Urine 544 c.c.]	,,	—	,, ,,
,, 6th ,,	—	[Urine 500 c.c.]	,,	—	—
,, 7th ,,	—	[Urine 1400 c.c.]	,,	—	—
,, 8th ,,	—	[Urine 840 c.c.]	,,	—	—
,, 9th ,,	—	[Urine 420 c.c.]	,,	—	—

It will be noticed that the chief alteration in the appearance of the casts observed during suppression of urine consisted in their large size, in the coarseness of the granules they contained, and in the frequent presence of an external epithelial envelope.

Suppression of urine in blackwater fever is known not to be necessarily complete, and hence the term oliguria is sometimes employed to denote this condition, instead of the more familiar terms 'suppression' or 'anuria.' A search of the literature has not enabled us to find exact records of the amounts of urine passed in individual cases during suppression, but two of our own cases (7a and 11) furnish data on this point. The daily amounts in these two cases are given in Table 41, and will be seen to range in Case 7a from 11 c.cm. to 43 c.cm., and in Case 11 from 8 c.cm. to 92 c.cm. The urine was voided naturally, but owing to the small quantities present in the bladder some difficulty was experienced in voiding it, and it is not certain that the bladder was always completely emptied. In any case, however, the daily variations were not usually considerable. The average amount of urine secreted by Case 7a was 28 c.cm. per day, and by Case 11 66 c cm. per day (Table 42).

TABLE 42. Average daily amount of urine passed during suppression of urine in blackwater fever. The colour of the urine was light yellow or amber.

Case	Period of suppression	Average amount of urine daily during suppression	Sp. gr. of urine	Coagulable proteid in urine
7a	Nine days	28 c.c.	1·010 to 1·015	$\frac{1}{4}$ col. to $\frac{2}{3}$ col.
11	Six days	66 c.c.	1·008 to 1·009 (on fourth and fifth days)	$\frac{2}{5}$ col. to $\frac{1}{2}$ col.

The urine was pale yellow or amber in colour, and was of markedly low specific gravity, namely, 1·010 to 1·015 in the first case and 1·008 to 1·009 in the second case. Unfortunately, no estimation of the amount of urea present could be made. Another striking feature of the urine was the large amount of coagulated proteid which it contained, the precipitate produced on acidifying and boiling measuring, on standing, $\frac{1}{4}$ col. to $\frac{2}{3}$ col. of the urine taken. In Table 41, together with the above two cases, in which death occurred on the tenth and ninth days respectively, another case is also recorded, because the question arose whether this case, in which death occurred on the ninth day, should be regarded as one of partial suppression. The average daily amount of urine was 640 c.cm. ($22\frac{1}{2}$ oz.), and its specific gravity was somewhat low, being 1·015. Unfortunately, as in the preceding cases, no estimation of the output of urea was possible. The coagulable proteid obtained on acidifying and boiling measured, on standing, $\frac{1}{10}$ col. Further reference will be made to this case later.

Attention must now be directed to the condition of the kidneys at the time of death from suppression of urine in Cases 7a and 11. Before proceeding further, however, reference may be made to the literature of this subject. The number of post-mortem records of the aspect of the kidneys in suppression of urine in blackwater fever, which are accompanied by a report of the microscopical appearances of these organs, is very small. Two cases of death with suppression of urine in blackwater fever are described by H. Werner,* death in one case (Case 1) taking place on the day of the attack, and in the other case (Case 3) on the third day after the commencement of haemoglobinuria. In the first case the kidneys were dark violet in colour and were not markedly enlarged. The medulla showed a dark brown striation to the naked eye. On microscopical examination a finely granular exudation was seen within the Malpighian capsules, and occasionally separated epithelial cells were noted, but the glomerular cells were unaltered. The epithelium of the convoluted and straight tubules was normal, but their lumen was enlarged and contained coagulated material exhibiting granules, which were fine near the glomerulus but became progressively coarser as the collecting tubules were approached. No trace of an inflammatory process in the connective tissue of the kidney was observed. Inside the blood vessels was seen black malarial pigment, and in some places plasmodial division forms. In the second case described by Werner the kidneys were of a pale brown to yellowish red colour, and were enlarged. The microscopical appearances were the same as in the first case, except that the epithelium of the convoluted tubules showed in places cloudy swelling.

The cases coming under our own observation were two in number (Cases 7a and 11, Tables 41 and 42; clinical histories, pp. 195 to 201 and pp. 217 to 222), death occurring, as already mentioned, on the tenth and ninth days respectively, the duration of suppression being eight days in the first case and five days in the second. The kidneys showed no obvious congestion, though somewhat dark in aspect. They were of a brownish colour. The capsule stripped readily in Case 7a, and with slight erosions in Case 11. A most striking feature of the kidneys was their marked enlargement, measuring in Case 7a 5in. in length by 3in. in breadth and $1\frac{1}{4}$in. in thickness; in Case 11 the enlargement was very nearly as great. The cortex could not be sharply differentiated from the medulla, so that it was not possible to say how far the degree of swelling was greater in the former as

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* Über die Nieren beim Schwarzwasserfieber, Arch. f. Schiffs- und Tropenhygiene, 1907, B. 11, S. 5.

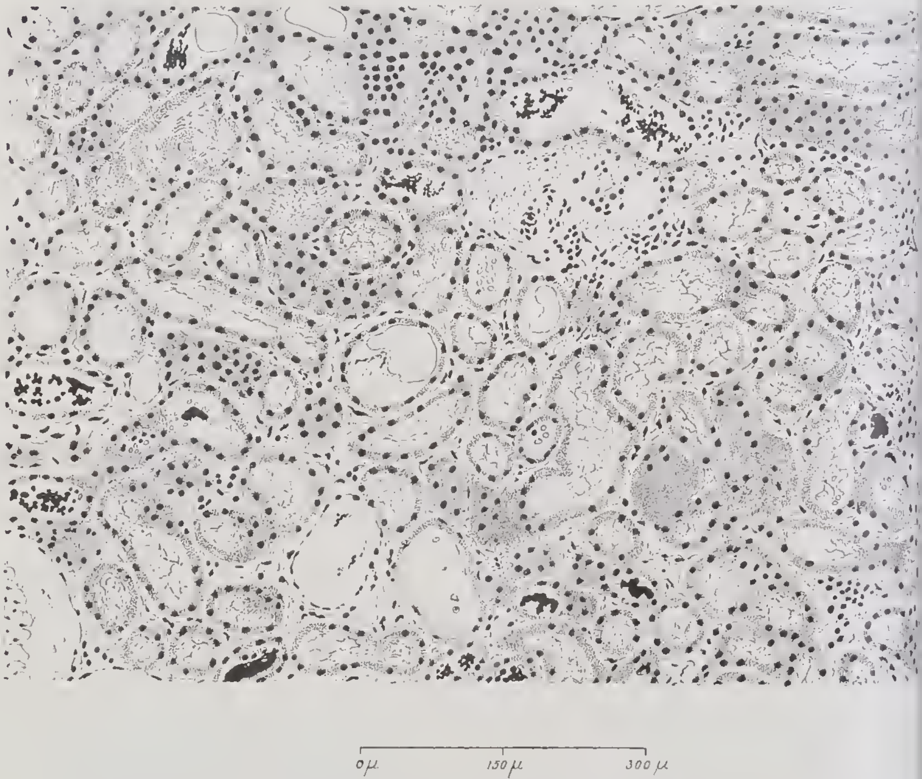

FIG. 33. Section of cortex of kidney during suppression of urine, eight days after haemoglobinuria had ceased. Blackwater Fever, Case 7a. The renal tubules are considerably distended with fluid. The lumen of thirteen of the tubles is partly occupied by darkly staining coarse granules measuring about 5μ in diameter, some of which are discrete, while others have coalesced into irregular masses. The epithelium of the tubules is flattened, but is not degenerated. The Malpighian capsules are dilated, but otherwise unaltered. The interstitial tissue, which seems to be also distended with fluid, exhibits no cell infiltration. No malarial pigment is recognisable. Hardened in alcohol. Stained with iron alum haematoxylin. × 120.

compared with the latter. A further characteristic alteration in the aspect of the kidney was presented by the medulla, which was quite abnormal, being dark brown almost black in colour, especially at the bases of the pyramids. On closer examination it was seen that this was due to fine brown radiating lines, presenting a somewhat fan-like character and readily seen with the naked eye. Fine brownish dots could also be recognised, though not so readily, scattered here and

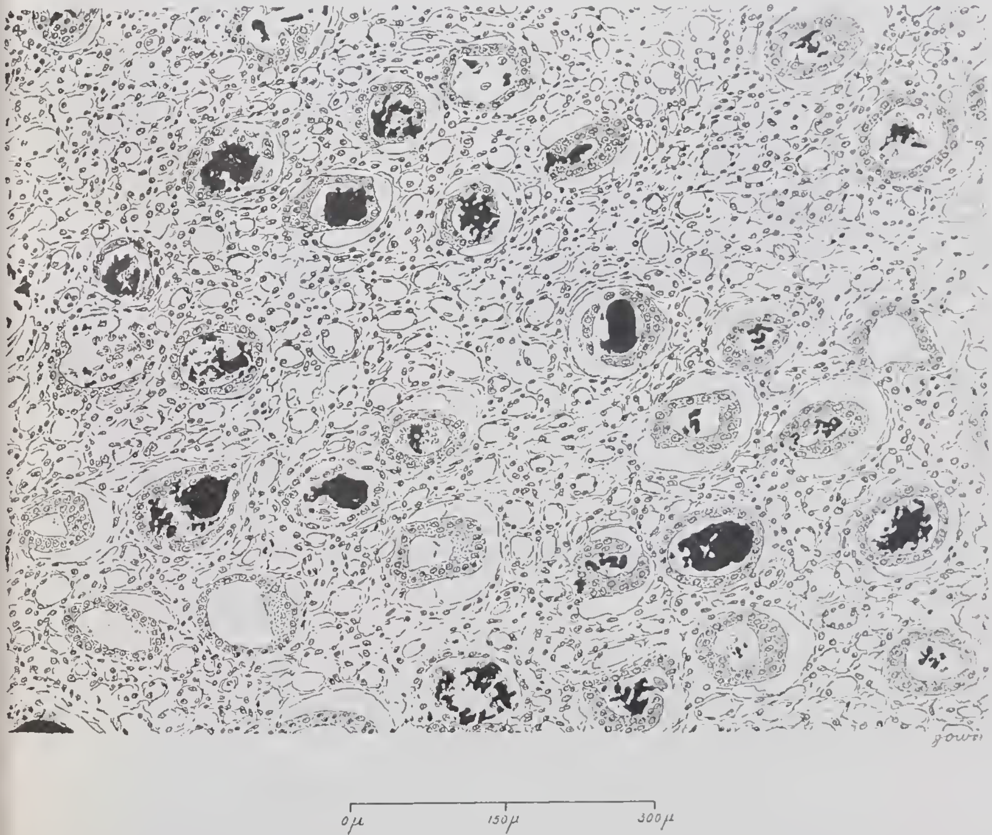

FIG. 34. Transverse section of medulla of kidney during suppression of urine, eight days after haemoglobinuria had ceased. Blackwater Fever, Case 7a. Many of the large collecting tubules, which are dilated and exhibit flattening of the epithelial lining, are partly filled with darkly staining granular material. The individual granules, which are about 5μ in diameter, are in some places discrete but are more frequently closely packed so as to form irregular masses. In some of the tubules also free epithelial cells are seen. The renal epithelium though flattened shows no degenerative changes. The interstitial tissue does not exhibit any cell infiltration or other marked alteration. No malarial pigment is recognisable. Hardened in alcohol. Stained with iron alum haematoxylin. × 130.

there throughout the cortex. The above two changes—the enlargement of the kidney and the distribution of the dark brown medullary striation and cortical stippling—are shown somewhat diagrammatically in Fig. 61, C. Recent haemorrhages of a punctate character were seen in Case 7a in the mucous membrane of the renal pelvis, as well as an ecchymosis about 1 cm. in diameter beneath the capsule of the left kidney.

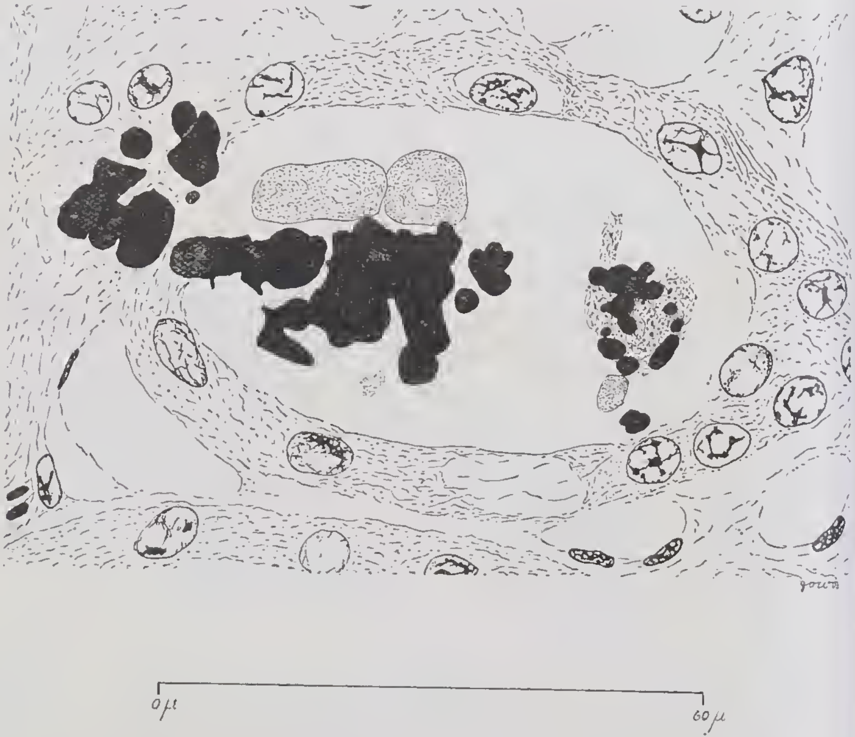

FIG. 35. Section of cortex of kidney during suppression of urine, eight days after haemoglobinuria had ceased. Blackwater Fever, Case 7a. A dilated convoluted tubule is seen in transverse section. The lumen of the tubule is partly occupied by irregular masses, composed of darkly stained granules. The individual granules, when discrete, range from 2μ or 3μ to 5μ in diameter. To the right some finely granular débris is seen. Within the lumen of the tubule are two free epithelial cells, the nuclei of which are indistinct. The epithelium of the wall of the tubule is flattened, but does not present any marked evidence of degeneration. Hardened in alcohol. Stained with iron alum haematoxylin. × 1350.

On making sections of the kidneys in Cases 7a and 11, it was found (Figs. 34, 36, 38, 40, 54 and 56)* that many, but not all, of the ducts of Bertini, the largest collecting tubules, contained dark reddish brown granular plugs 40μ to 80μ in transverse measurement, similar to those already described in the urine (Fig. 32), and giving rise to the brown radiation, already mentioned, visible to the naked eye

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* Figs. 53–60 are opposite p. 131 (Plates 1 and 2).

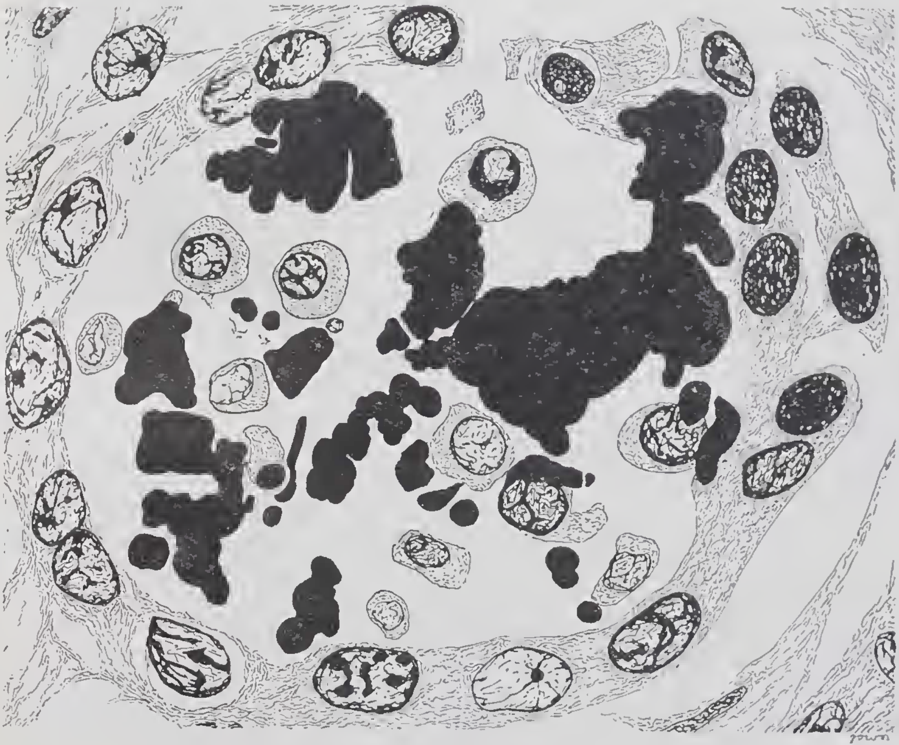

FIG. 36. Section of medulla of kidney during suppression of urine, eight days after haemoglobinuria had ceased. Blackwater Fever, Case 7a. A collecting tubule is shown in transverse section. The lumen of the tubule is partly filled with free epithelial cells and irregular masses composed of darkly stained granules of varying size, which have for the most part coalesced. The size of the individual granules ranges from 3μ to 5μ. Some of the free epithelial cells contained within the tubule exhibit a well stained nucleus, the cytoplasm of the cell not being markedly altered in appearance; in others the cytoplasm is stained more darkly than usual and the nuclei are indistinct. Hardened in alcohol. Stained with iron alum haematoxylin. × 1150.

in the medulla of the kidney. In places these plugs had been detached, sometimes, but not always, together with the epithelial lining of the collecting tubule, the basement membrane being then bare. In some cases a granular plug, covered externally with epithelium, had been detached at a higher level and was found partly filling a duct of Bertini. In the smaller straight tubules similar plugs of lesser dimensions, from 30μ to 40μ in diameter, were frequently seen. In the uriniferous tubules (Figs. 33, 35, 37, 39, 53 and 55) granular plugs or casts were sometimes present, frequently absent.

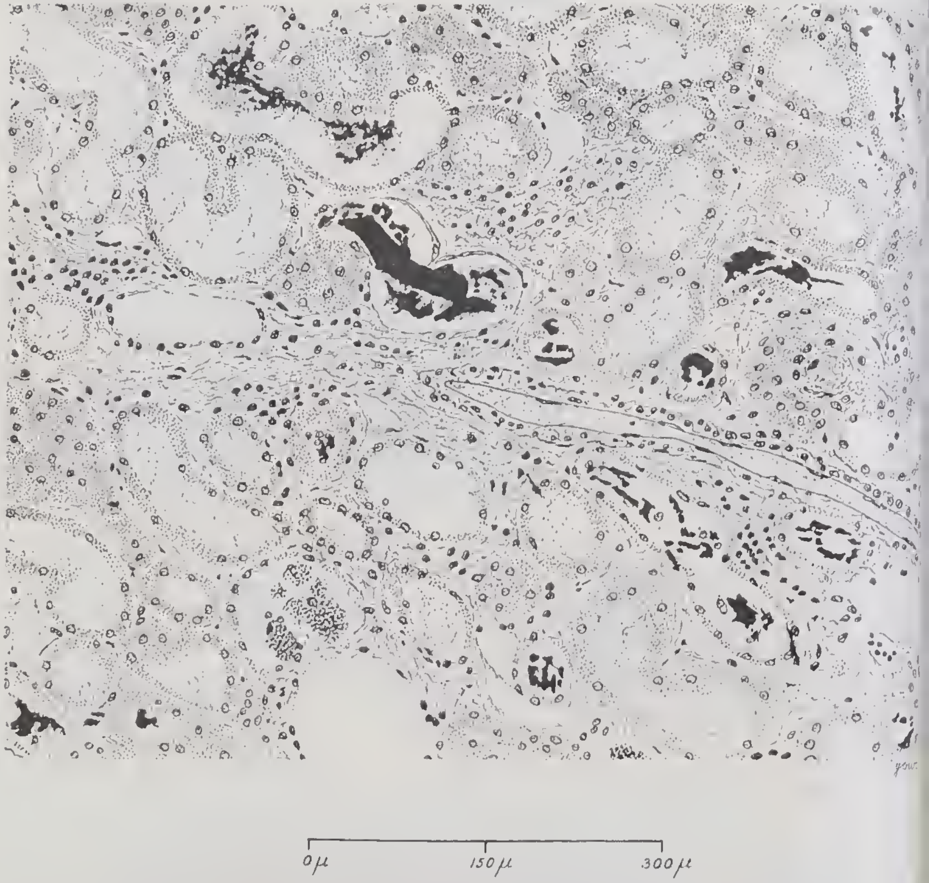

FIG. 37. Section of cortex of kidney during suppression of urine, five days after haemoglobinuria had ceased. Blackwater Fever, Case 11. The renal tubules are generally dilated to a considerable degree, the epithelium being in many places correspondingly thinned. In thirteen of the tubules dense black material is seen partly filling the lumen. In two situations this material is observed to consist of more or less discrete granules, while elsewhere the individual granules are indistinct. A small amount of flocculent material, with here and there a nucleus, can be recognised in some of the tubules. In one tubule the epithelium has disappeared from part of the wall of the tubule. The renal epithelium does not appear to be degenerated. The interstitial tissue is distended with fluid, but does not exhibit any cell infiltration. The blood vessels are not congested. Few red cells are seen. No malarial pigment is recognisable. Hardened in alcohol. Stained with iron alum haematoxylin. × 150.

These plugs, which varied considerably in diameter, ranging from 30μ to 80μ, gave rise to the brown stippling sometimes seen on a cut surface of the cortex of the kidney (Fig. 61).

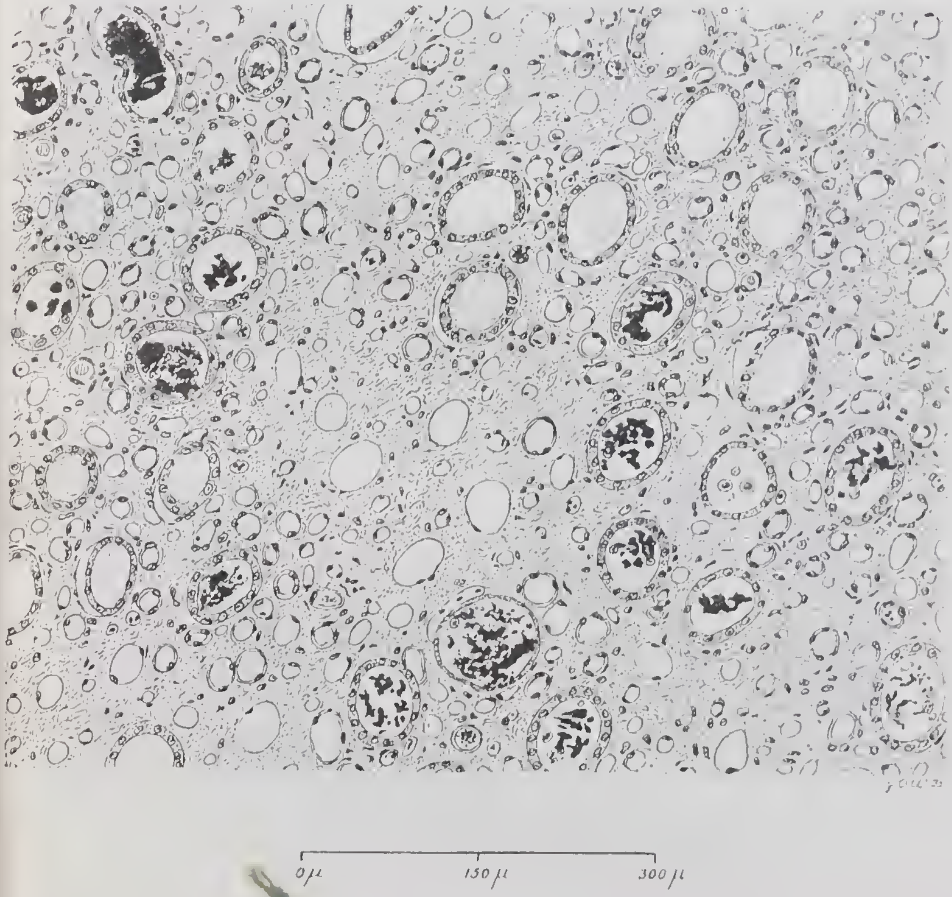

FIG. 38. Transverse section of medulla of kidney during suppression of urine, five days after haemoglobinuria had ceased. Blackwater Fever, Case 11. About half of the collecting tubules are partly occupied by darkly staining material, which in some places appears uniform in aspect, but elsewhere when not very dense is seen to be made up of granules of varying size. In addition epithelial cells can be recognised in six of the collecting tubules. The collecting tubules are more or less distended and their epithelial lining is correspondingly thinned. The epithelium of none of the collecting tubules has been shed. The renal epithelium does not exhibit degenerative changes. The blood capillaries are with very few exceptions empty, no red cells being seen. The interstitial tissue is not markedly altered in appearance. No malarial pigment is recognisable. Hardened in alcohol. Stained with iron alum haematoxylin. × 155.

The renal plugs were seen in sections stained with haematoxylin to be very irregular and heterogeneous in aspect, not as a rule completely filling the lumen of the tubule. They were in part made up of granules, readily taking up the dye so as to be black in colour, and of irregular masses, measuring, as much as 50μ or more across, formed by the coalescence of the granules. In addition to these granules other elements were often present, namely, detached epithelial cells and smaller lightly stained granules, together with flocculent matter.

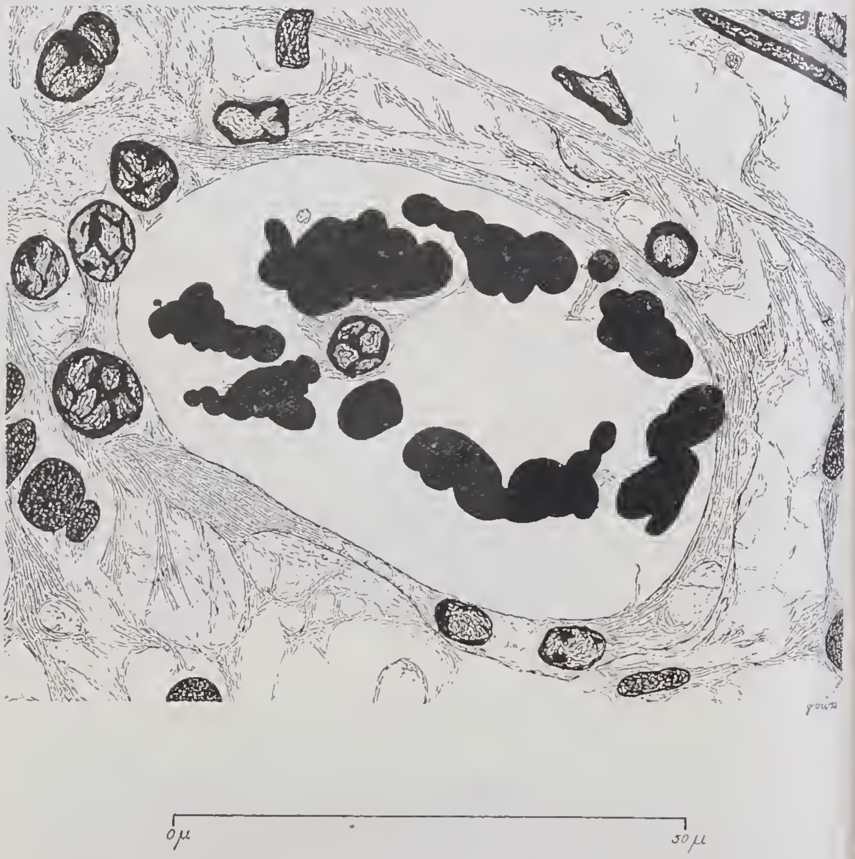

FIG. 39. Section of cortex of kidney during suppression of urine, five days after haemoglobinuria had ceased. Blackwater Fever, Case 11. A dilated renal tubule is seen in transverse section. The lumen of the tubule is partly occupied by darkly stained granules, varying in diameter from 3μ to about 5μ, which have joined together so as to form irregular more or less elongated masses. In addition one free epithelial cell is seen among the granular masses. The epithelial lining of the tubule can be recognised without difficulty, it is thinned, especially above. Except for this thinning, the result of dilation of the tubule, the epithelium does not seem otherwise altered. Hardened in alcohol. Stained with iron alum haematoxylin. × 1300.

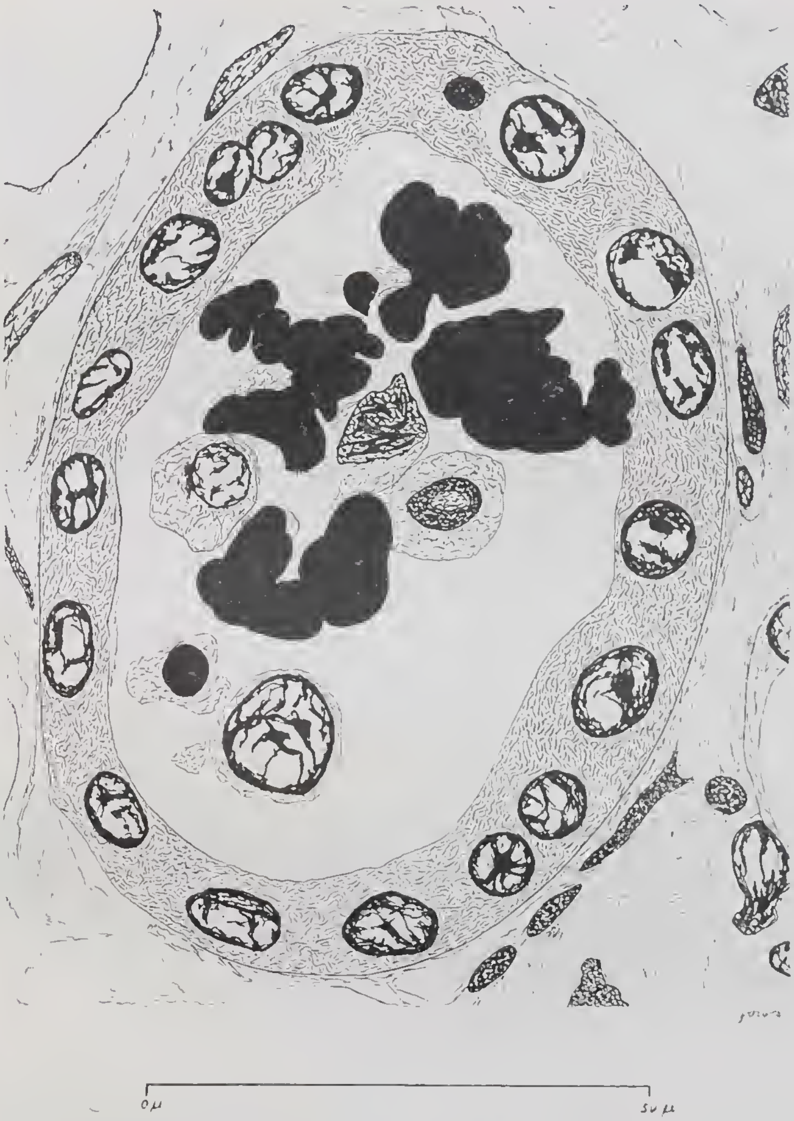

FIG. 40. Section of medulla of kidney during suppression of urine, five days after haemoglobinuria had ceased. Blackwater Fever, Case 11. Transverse section of a collecting tubule. The lumen of the tubule is incompletely filled with epithelial cells and darkly stained granular material, the individual granules, which are from 3μ to 5μ in diameter, having coalesced so as to form irregular groups. The nuclei of two of the free epithelial cells, contained in the lumen of the tubule present a shrunken aspect and do not exhibit a well formed chromatin network. The epithelial cells lining the tubule do not exhibit any marked change, and the interstitial tissue is free from any indication of cell infiltration. Hardened in alcohol. Stained with iron alum haematoxylin × 1300.

The individual darkly stained granules were usually of large size, measuring 3μ to 5μ in diameter. They were of the same size in the cortex as in the medulla, not presenting any increase in size as the renal pelvis was approached. A few tubules were met with, both in the cortex and in the medulla, containing much smaller granules, measuring from 2μ to less than $0{\cdot}5\mu$ in diameter (cp. Fig. 37). These were sometimes very dark, almost black in colour, sometimes less deeply stained, occasionally merely lightly tinted.

Epithelial cells were a common feature of the plugs (Figs. 37 to 40). They were of large size, with abundant cytoplasm. The nucleus was sometimes darkly, sometimes lightly stained, or had contracted to form a small darkly stained mass, the chromatin network of which was not well defined. The cytoplasm usually stained more deeply with acid dyes than did that of the epithelial cells lining the tubule, but was less granular in aspect than the latter.

The lightly stained débris sometimes seen in the tubules in addition to the above elements was in part made up of well-defined fine granules, 2μ to 5μ or less in diameter, and in part of amorphous flocculent material. Every transition could be observed between the former and the darkly stained granules of smaller size described above.

The most striking feature of the material contained in the lumen of the renal tubules was its multiformity and irregularity, which was much greater than that observed in the renal casts seen in the urine during experimental haemoglobinuria or during blackwater (Figs. 21 to 32).

The uriniferous tubules were considerably distended, the lumen of the convoluted tubules commonly having a diameter of 40μ to 50μ (Figs. 33 to 40), and the epithelial lining affected being correspondingly flattened. The same distension affected also the cavity of the Malpighian capsules (Fig. 33), which occasionally contained lightly stained finely granular deposit. Beyond flattening, however, no other abnormal change was recognisable in the epithelial cells of the kidney. The nuclear staining was unaltered, and cloudy swelling was not observed. No brown granules were met with in the epithelial cells of the uriniferous tubules.

In the interstitial tissue of the kidney no cell infiltration or other change was present. The blood vessels of the kidney were usually empty. No malarial pigment was found in the blood vessels or elsewhere. No haemorrhages were seen in the interstitial tissue of

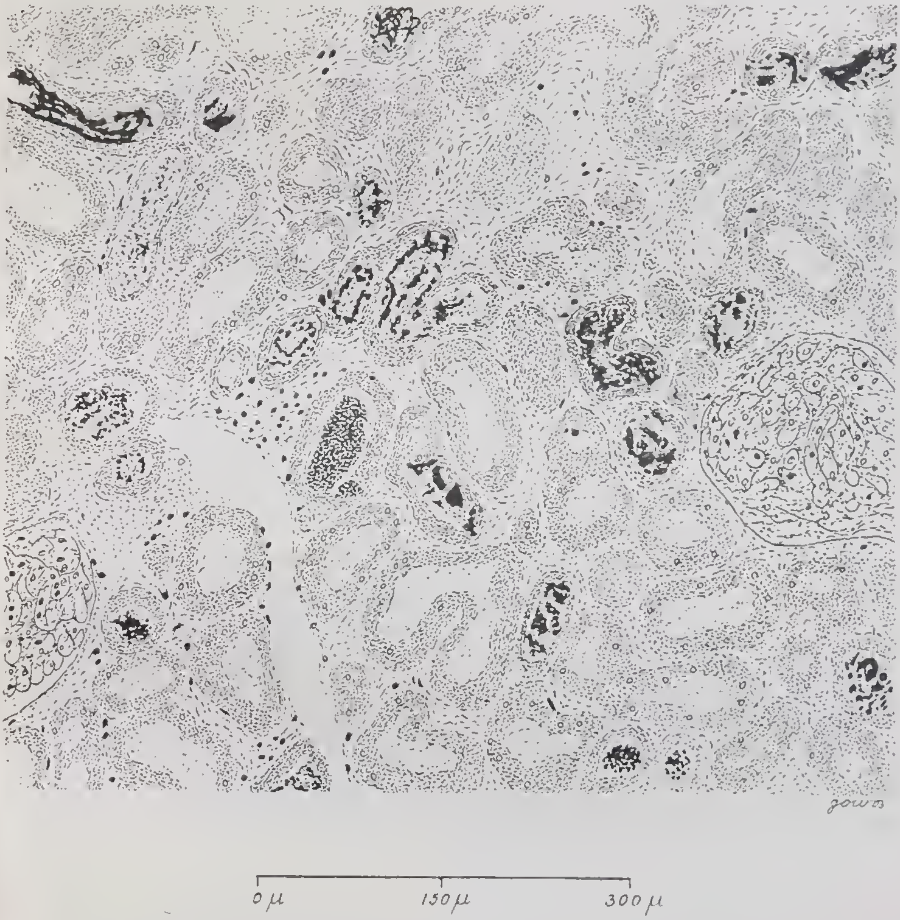

FIG. 41. Section of cortex of kidney, seven days after haemoglobinuria had ceased. Blackwater Fever, Case 16. Many of the larger renal tubules (mostly convoluted tubules) and some of the smaller contain darkly staining material, partially filling the lumen. This material is in part so darkly stained as to look homogeneous, but where less dense it can be recognised to be made up of irregular granules of varying size. The epithelium of the renal tubules, which in this section is very faintly stained, does not present any unmistakable evidence of degeneration. The interstitial tissue does not exhibit any marked increase in amount, nor any cell infiltration. No malarial pigment can be recognised. Fixed in Flemming's solution. Stained with iron alum haematoxylin. × 160.

either cortex or medulla, but haemorrhage beneath the capsule o one kidney, already referred to, was observed in Case 7a.

The position and aspects of the renal casts, and the distension of the renal tubules and Malpighian capsules are represented diagrammatically in Fig. 62A.

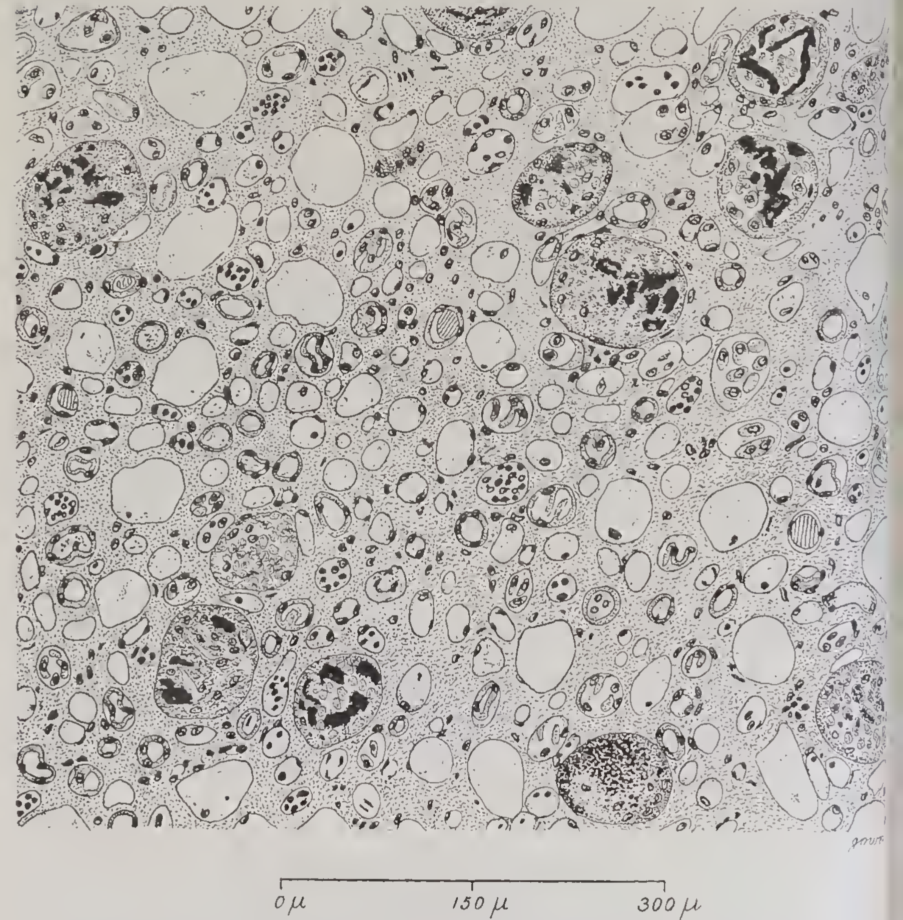

FIG. 42. Transverse section of medulla of kidney, seven days after haemoglobinuria had ceased. Blackwater Fever, Case 16. Many of the collecting tubules are in part occupied by darkly staining material, usually dense in aspect, but in one of the tubules coarse granules are seen. In some of these tubules epithelial cells and débris can also be recognised. Some of the collecting tubules are devoid of epithelial lining and contain a very small amount of flocculent material. Four of the smaller tubules show hyaline casts, indicated by parallel shading. A few blood cells are seen containing red cells darkly stained. The interstitial tissue shows no increase in amount nor is any cell infiltration observable. No malarial pigment can be seen. Hardened in alcohol. Stained with iron alum haematoxylin. × 160.

We will now refer to the histological changes presented by the kidneys in another attack of blackwater fever (Case 16), in which death occurred seven days after haemoglobinuria had ceased, during which period 640 c.cm. (22½ oz.) of urine of specific gravity 1·015 were passed daily (see clinical notes, pp. 235 to 239). Although in this case it

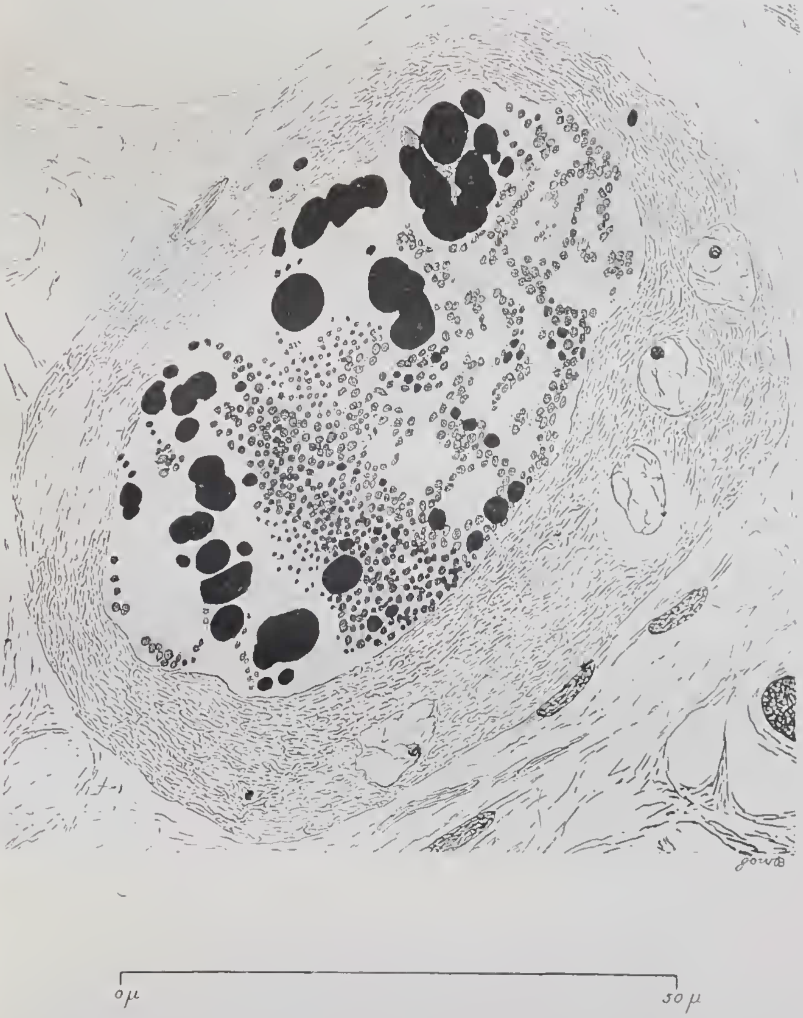

FIG. 43. Section of cortex of kidney, seven days after haemoglobinuria had ceased. Blackwater Fever, Case 16. Transverse section of renal tubule. The lumen is occupied by darkly stained large coarse granules, 3μ to 5μ in diameter, similar to those seen in the preceding Figures, and also by smaller granules, less darkly stained and about 1·5μ in diameter. The section is lightly stained. Fixed in Flemming's solution. Stained with iron alum haematoxylin × 1400.

is not certain that some degree of suppression of urine occurred, the condition of the kidneys offers an interesting comparison with that found in Cases 7a and 11.

In naked eye appearance the kidneys presented little change beyond slight enlargement. Sections of the kidneys (Figs. 41-44 and 57-58) were, however, very similar to those of the preceding two cases. Many of the uriniferous and collecting tubules contained

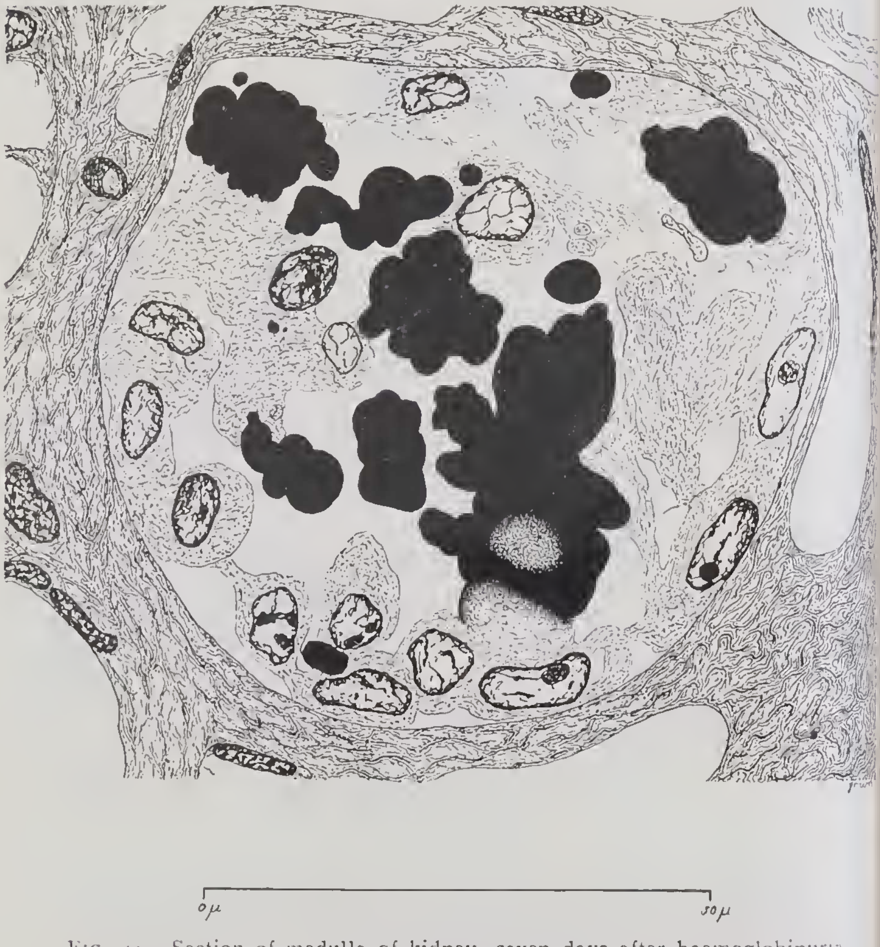

FIG. 44. Section of medulla of kidney, seven days after haemoglobinuria had ceased. Blackwater Fever, Case 16. A large collecting tubule is shown in transverse section. In the lumen of the tubule are darkly stained coarse granules, 2μ to about 5μ in diameter, and masses formed by the coalescence of these granules. Free epithelial cells are also seen within the tubule, among these masses. The cells of the epithelial lining of the tubule are partially detached. Fixed in Flemming's solution. Stained with iron alum haematoxylin. × 1300.

large darkly staining granules and masses composed of groups of granules more or less completely fused together. These granules and granular masses were accompanied by epithelial cells lying free in the lumen of the tubules, and also by a variable amount of lightly staining granular and flocculent material. The individual darkly staining granules were usually of large size, ranging from 3μ to 5μ in diameter, and in addition collections of darkly staining granules of much smaller size, measuring 2μ to less than 0·5μ in diameter, were frequently met with (Figs. 43 and 57). The lumen of the tubules generally contained numerous epithelial cells. Throughout the kidneys the epithelial cells lining the tubules were more or less

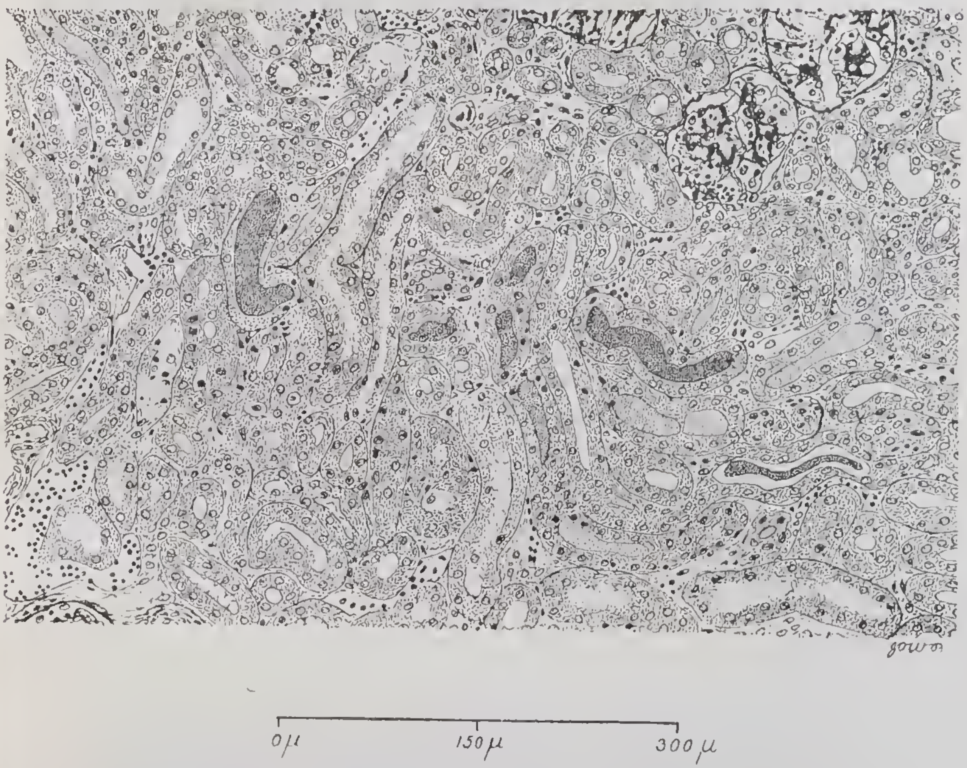

FIG. 45. Section of cortex of kidney during experimental haemoglobinuria. Rabbit 7, Table 35. In some of the tubules dark masses containing granules are seen. The individual granules are small and vary in number, being generally somewhat scanty. The renal epithelium is healthy and the tubules are not dilated. The interstitial tissue shows no change. Red cells are recognisable in several intertubular capillaries and in one Malpighian capsule. Hardened in alcohol. Stained with iron alum haematoxylin. × 170.

completely detached (Fig. 44). Some of the tubules, in particular the large collecting tubules, exhibited no epithelial covering, the basement membrane being bare (Fig. 42). Lightly staining material, present in the form of granules, $1{\cdot}5\,\mu$ to $0{\cdot}5\,\mu$ in diameter, and of flocculent deposit, is seen in Figs. 43 and 58. The lumen of the

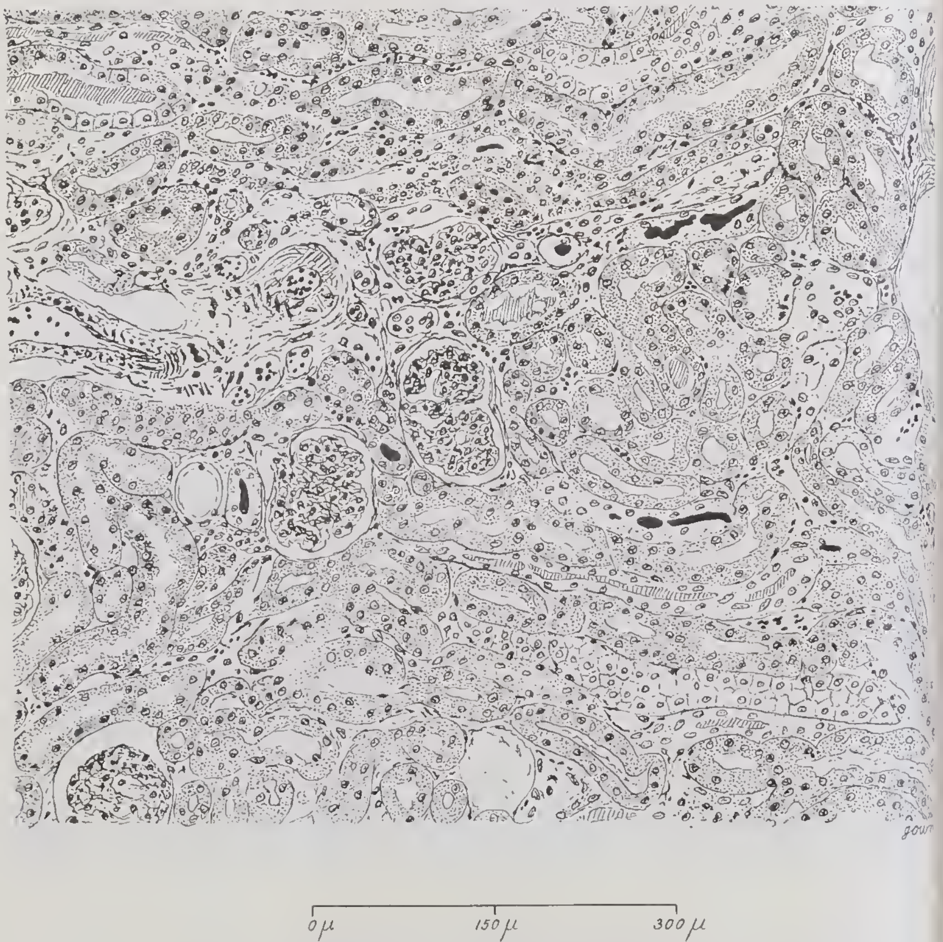

FIG. 46. Another section of cortex of kidney during experimental haemoglobinuria of Rabbit 7, Table 35. The epithelium is healthy in aspect, showing no sign of cloudy swelling or other indication of degeneration. The tubules are not recognisably dilated, but casts of granular material, stained very darkly, are present in six of the tubules. Casts presenting a homogeneous aspect, indicated in the sketch by parallel shading, are seen in five tubules. There is no increase of nuclei or other change in the interstitial substance of the kidney. A few of the blood capillaries contain red cells, stained black. No malarial pigment is seen. Fixed in Flemming's solution. Stained with iron alum haematoxylin. × 155.

tubules was wide, but there was not the marked distension with flattening and thinning of the epithelial lining seen in the two preceding cases. The interstitial tissue showed no cell infiltration, nor was any recognisable increase of connective tissue present. No evidence of renal haemorrhage was found, and the blood vessels were free from malarial pigment. It will thus be seen that the chief points of difference, as regards the condition of the kidneys, between this case (16) and the preceding cases (7a and 11), in which suppression of urine occurred, are: the absence of distension of the renal tubules in degree sufficient to cause flattening and thinning of

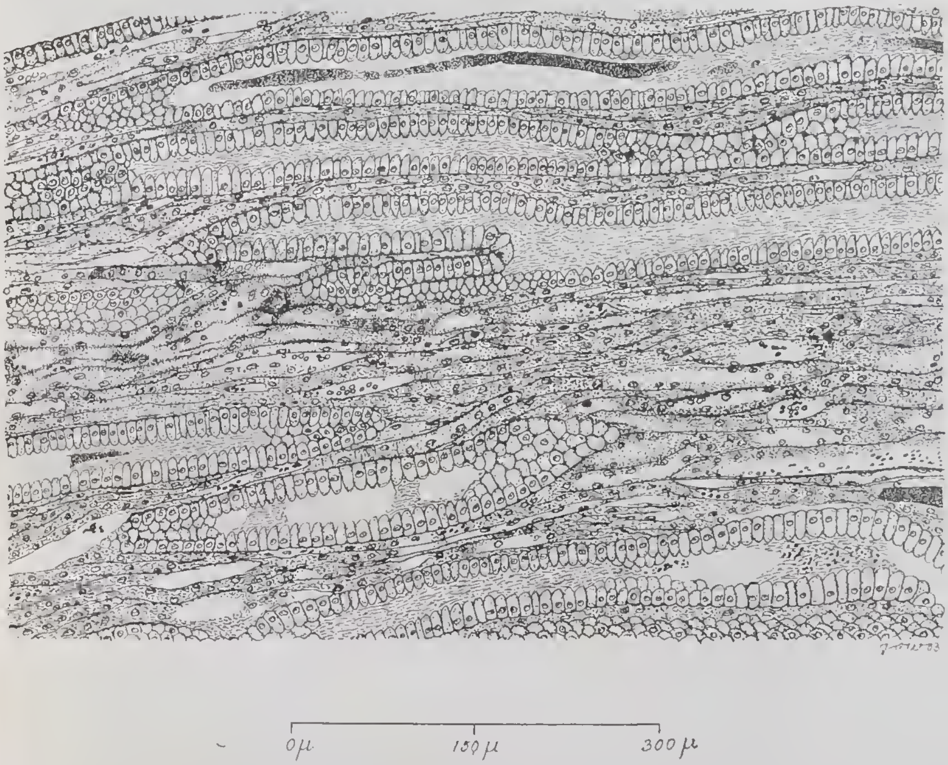

FIG. 47. Longitudinal section of medulla of kidney during experimental haemoglobinuria. Rabbit 7, Table 35. In four of the large collecting tubules granular casts are seen partly occupying the lumen of these tubules. The granules are fine, but vary somewhat in size and also in number in different situations. In addition some slightly staining flocculent material is observable in the tubules. The renal epithelium is healthy and the tubules are not dilated. No change is present in the interstitial tissue. A few of the blood capillaries contain red cells darkly stained. Hardened in alcohol. Stained with iron alum haematoxylin. × 170.

the epithelial lining; the frequency with which small granules, 2μ to less than $0{\cdot}5\mu$ in diameter, were met with; and the much greater extent to which the renal epithelial cells were loosened and detached.

The condition of the kidneys of the rabbit in experimental haemoglobinuria was also studied. In illustration of the changes observed, we give sketches of sections made: during haemoglobinuria (eighty-five minutes after the injection of dissolved haemoglobin, Experiment 7, Table 35), Figs. 45-49 and 59-60; twenty-two hours after injection and fourteen hours after cessation of haemoglobinuria (Experiment 9, Table 35), Figs. 50-51.

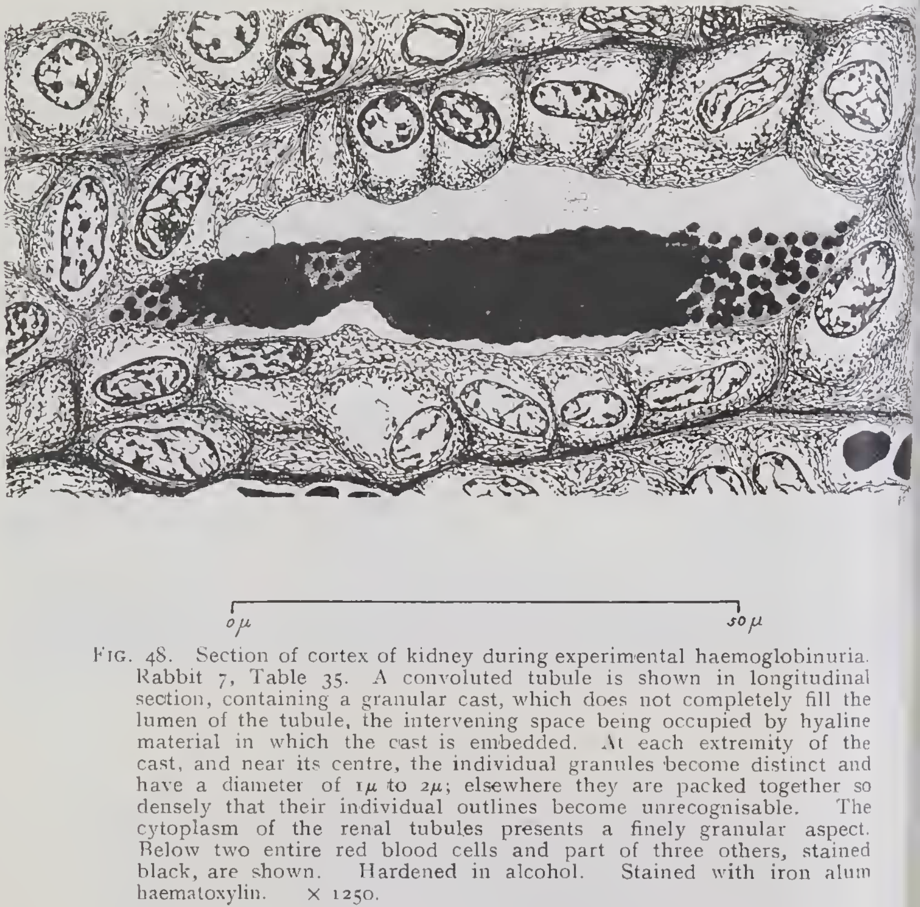

FIG. 48. Section of cortex of kidney during experimental haemoglobinuria. Rabbit 7, Table 35. A convoluted tubule is shown in longitudinal section, containing a granular cast, which does not completely fill the lumen of the tubule, the intervening space being occupied by hyaline material in which the cast is embedded. At each extremity of the cast, and near its centre, the individual granules become distinct and have a diameter of 1μ to 2μ; elsewhere they are packed together so densely that their individual outlines become unrecognisable. The cytoplasm of the renal tubules presents a finely granular aspect. Below two entire red blood cells and part of three others, stained black, are shown. Hardened in alcohol. Stained with iron alum haematoxylin. × 1250.

In each case the naked eye aspect of the kidneys presented no obvious change from the normal, the colour of the kidney being unaltered, the pyramid natural in appearance, and no brown stippling being recognisable. On cutting sections of the first kidney (Experiment 7, Table 35), the only change observable was the occasional presence, in some of the renal tubules, of granular material having the aspect of the casts already described in the urine (Figs. 21-24). This appeared to be scattered here and there in all parts of the uriniferous and straight tubules, but was absent from the Malpighian capsules. The granules varied in size from 2μ to less than $0{\cdot}5\mu$;

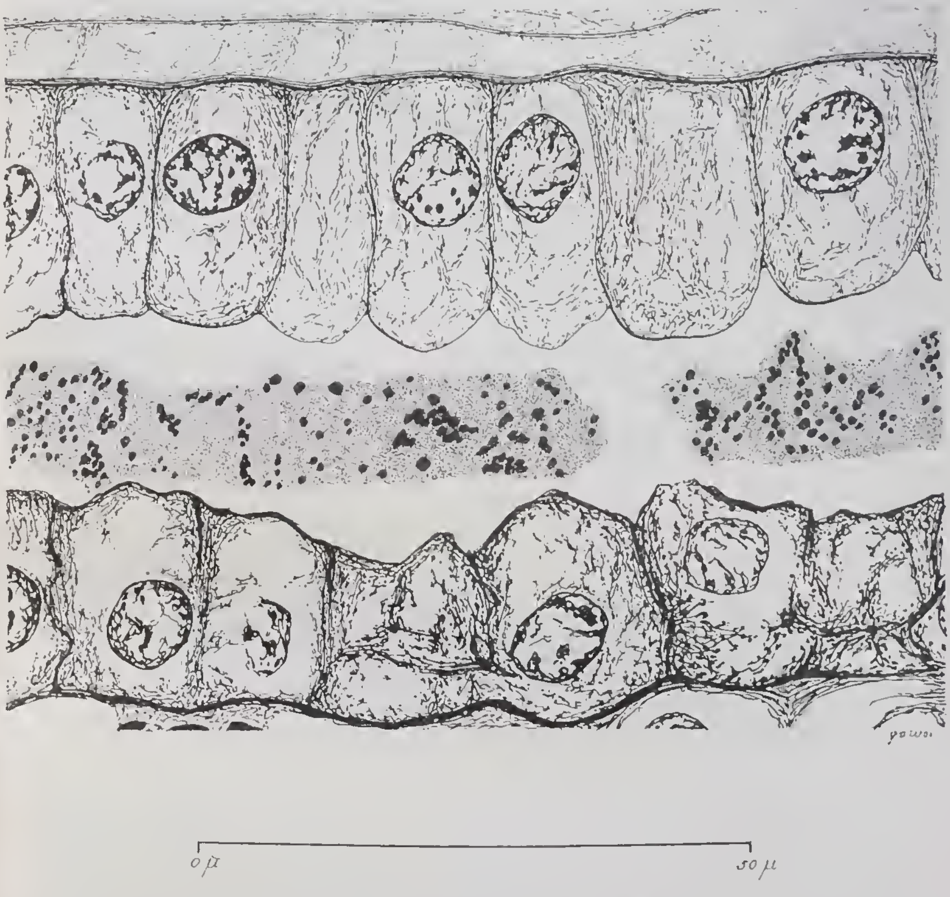

FIG. 49. Section of medulla of kidney during experimental haemoglobinuria. Rabbit 7, Table 35. In longitudinal section a collecting tubule is shown, partly occupied by a cast consisting of semi-opaque flocculent material containing here and there darkly stained granules $0{\cdot}5\mu$ to 1μ in diameter. The granules are mostly discrete; in some places they are in contact, but they do not appear to have undergone fusion. The renal epithelium is healthy in aspect. Hardened in alcohol. Stained with iron alum haematoxylin. × 1400.

they were brown in colour, in the unstained condition, and did not appear to increase in size as the pelvis of the kidney was approached. They stained readily, but were more easily decolourised than the larger granules seen in the kidneys in Cases 7a and 11. The casts varied in denseness, and the hyaline material which formed their

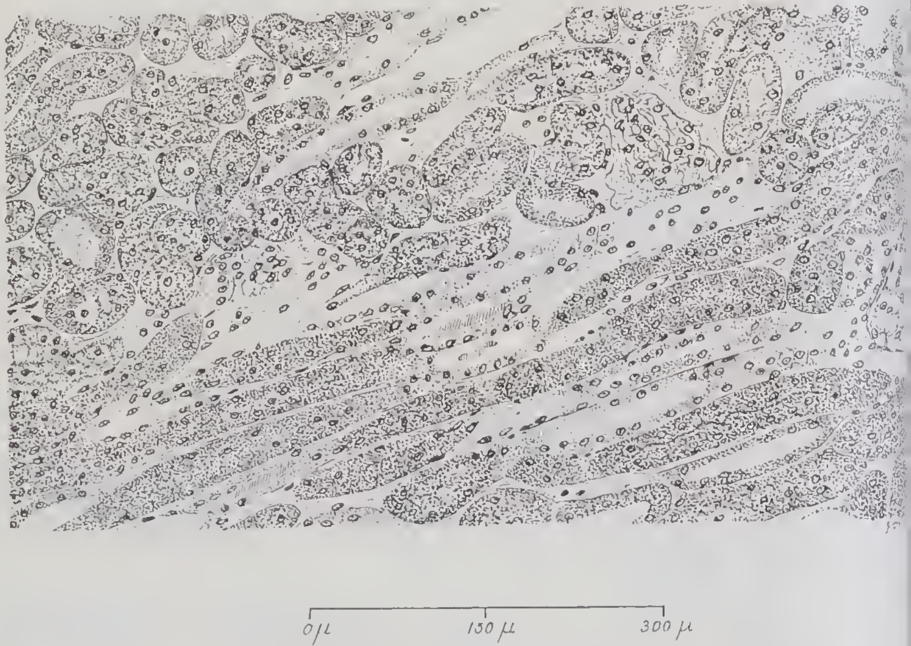

FIG. 50. Section of cortex of kidney fourteen hours after experimental haemoglobinuria. Rabbit 9, Table 35. The section shows portions of three Malpighian bodies and a number of tubules, chiefly convoluted tubules, all of which are normal in aspect, the epithelium being unaltered. Three of the smaller tubules show casts of hyaline material, but no darkly-staining granules are to be seen in any of the tubules. None of the tubules are dilated Fixed in Flemming's solution. Stained with iron alum haematoxylin. × 155.

basis contained sometimes few, sometimes numerous granules. Most of the casts were surrounded by hyaline material (Figs. 48 and 49). The lumen of some of the cortical tubules was filled with hyaline material which resembled haemoglobin, inasmuch as it readily took up acid dyes (Figs. 45 and 46). No change was seen in the blood vessels. The renal epithelium was perfectly normal in aspect.

The condition of the kidneys during experimental haemoglobinuria is represented diagrammatically in Figs. 61B and 62B.

Similar renal plugs were observed in cattle and dogs during red water due to piroplasmosis (Fig. 52).

The kidneys of the rabbit fourteen hours after the cessation of haemoglobinuria were, however, in all respects normal, no granular material being present in the tubules (Figs. 50 and 51).

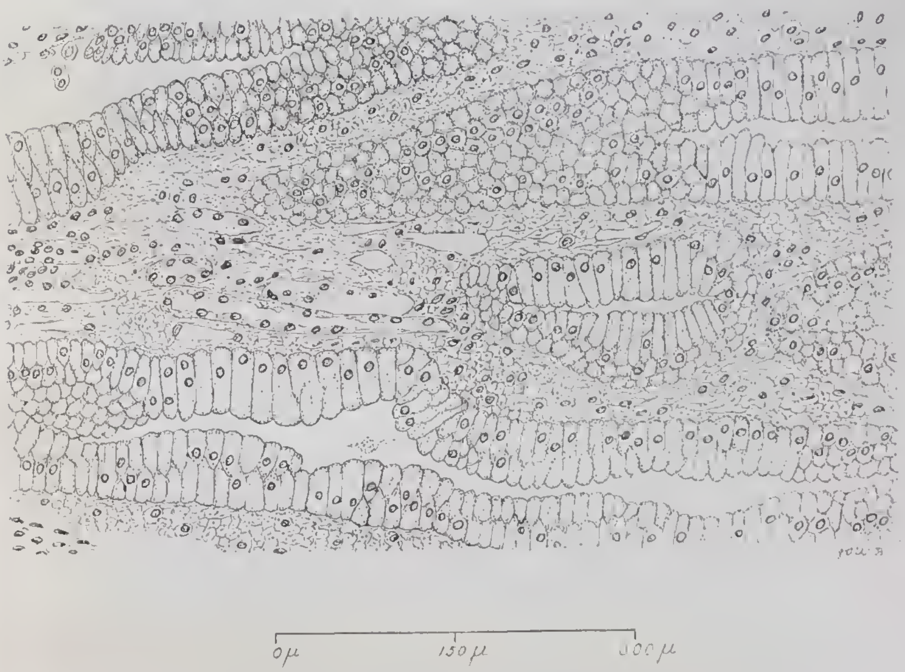

FIG. 51. Longitudinal section of medulla of kidney fourteen hours after experimental haemoglobinuria. Rabbit 9, Table 35. Three collecting tubules are seen, also several small tubules. The epithelium is everywhere healthy in aspect. A small amount of deposit is seen in the lumen of some of the tubules, but no darkly-staining material. There is no dilatation of the tubules, and the interstitial tissue is normal in aspect. Fixed in Flemming's solution. Stained with iron alum haematoxylin. × 155.

In the experiments upon rabbits, already described (Tables 35, 37), in which haemoglobinuria was produced by the intravenous injection of dissolved haemoglobin, an increased flow of urine occurred. Up to the present we have not obtained suppression of urine after injection. It will be, however, of interest to refer at this point to a group of experiments carried out by different observers, in which haemoglobinuria has been followed by anuria. In these experiments haemoglobinuria was produced by the injection of a haemolytic serum (Ponfick,[*] Joannovicz[†]) of a solution of potassium chlorate (Marchand,[‡] Lebedeff[§]), of glycerin (Lebedeff,[§] Afanassiew[||]), of iodine (Lebedeff,[§] Afanassiew[||]), of pyrogallic acid (Afanassiew[||]), and of toluylendiamin (Afanassiew[||]). By all these agents

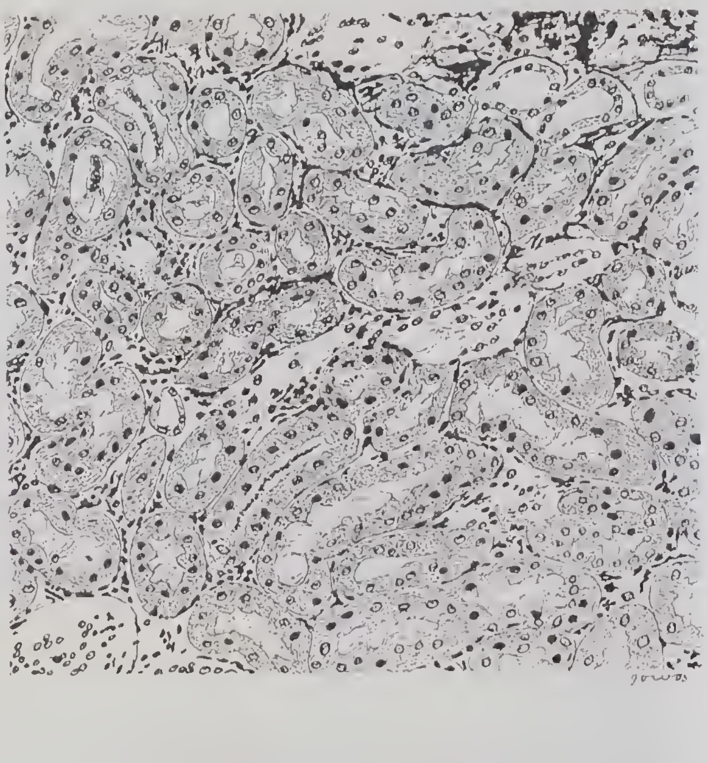

FIG. 52. Section of cortex of kidney of bull during redwater due to piroplasmosis. In three of the convoluted tubules casts with coarse irregular granules, darkly stained, are seen partly filling the lumen of the tubules. The epithelium is not degenerated and the tubules are not dilated. No alteration of the interstitial tissue is recognisable. Fixed in Flemming's solution. Stained with iron alum haematoxylin. × 170.

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[*] Experimentalle Beiträge zur Lehre von der Transfusion, Virchow's Arch., 1875, B. 62, S. 273.

[†] Experimentelle Untersuchungen über Ikterus, Zeitschr. f. Heilkunde, 1904, B. 25, S. 25.

[‡] Über die Intoxication durch chlorsaure Salze, Virch. Arch., 1879, Bd. 77, S. 455.

[§] Zur Kenntniss der feineren Veränderungen der Nieren bei Hämoglobinausscheidung, Virch. Arch., 1883, B. 91, S. 267.

[||] Über die pathologisch-anatomischen Veränderungen in den Nieren und in der Leber bei einigen mit Hämoglobinurie oder Ikterus verbundenen Vergiftungen, Virch. Arch., 1884, B. 98, S. 460.

haemoglobinuria may be obtained and sometimes anuria also. In the latter case the kidneys and spleen have been found enlarged, and the former has shown dark medullary striation and brown cortical stippling, while the uriniferous tubules have contained plugs of dark brown granular material, the condition being similar to that already

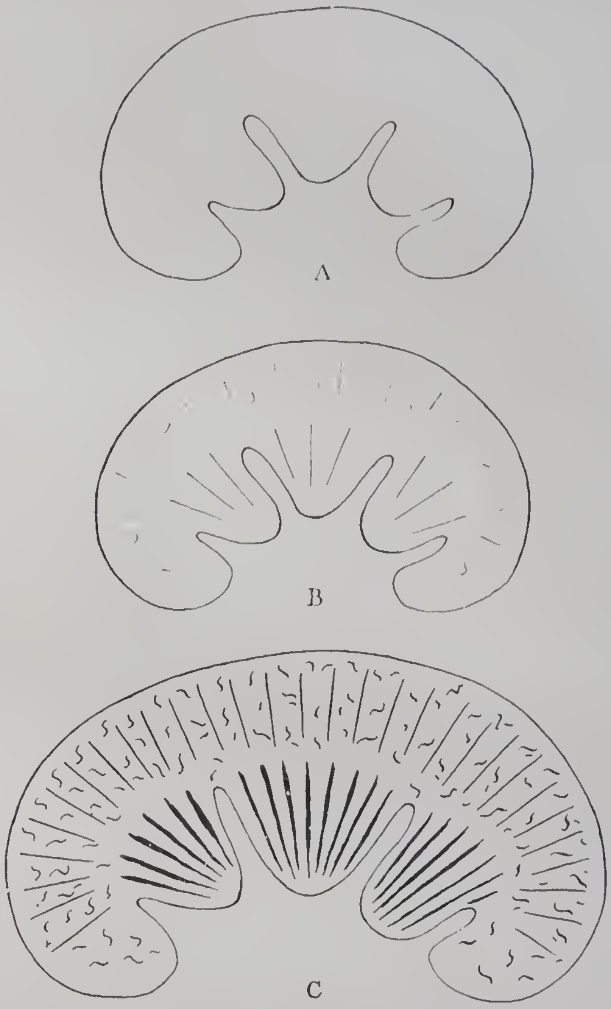

Fig. 61. Diagrammatic representation of the condition of the kidney in health (A), during experimental haemoglobinuria of rabbit (B), and in suppression of urine following upon blackwater fever (C). In B small casts are present in some of the uriniferous and collecting tubules; in C large casts are present in many of the uriniferous and collecting tubules. In the latter considerable enlargement of the kidney is also observed. For the sake of comparison B is represented as tri-papillary instead of being uni-papillary, as in the rabbit.

described as occurring in suppression of urine in blackwater fever. The condition of the animals, however, differed in several important particulars from that obtaining in blackwater fever, for, in addition to the profound toxic effect produced by the agents employed, marked changes in the appearance of the red blood cells such as have not

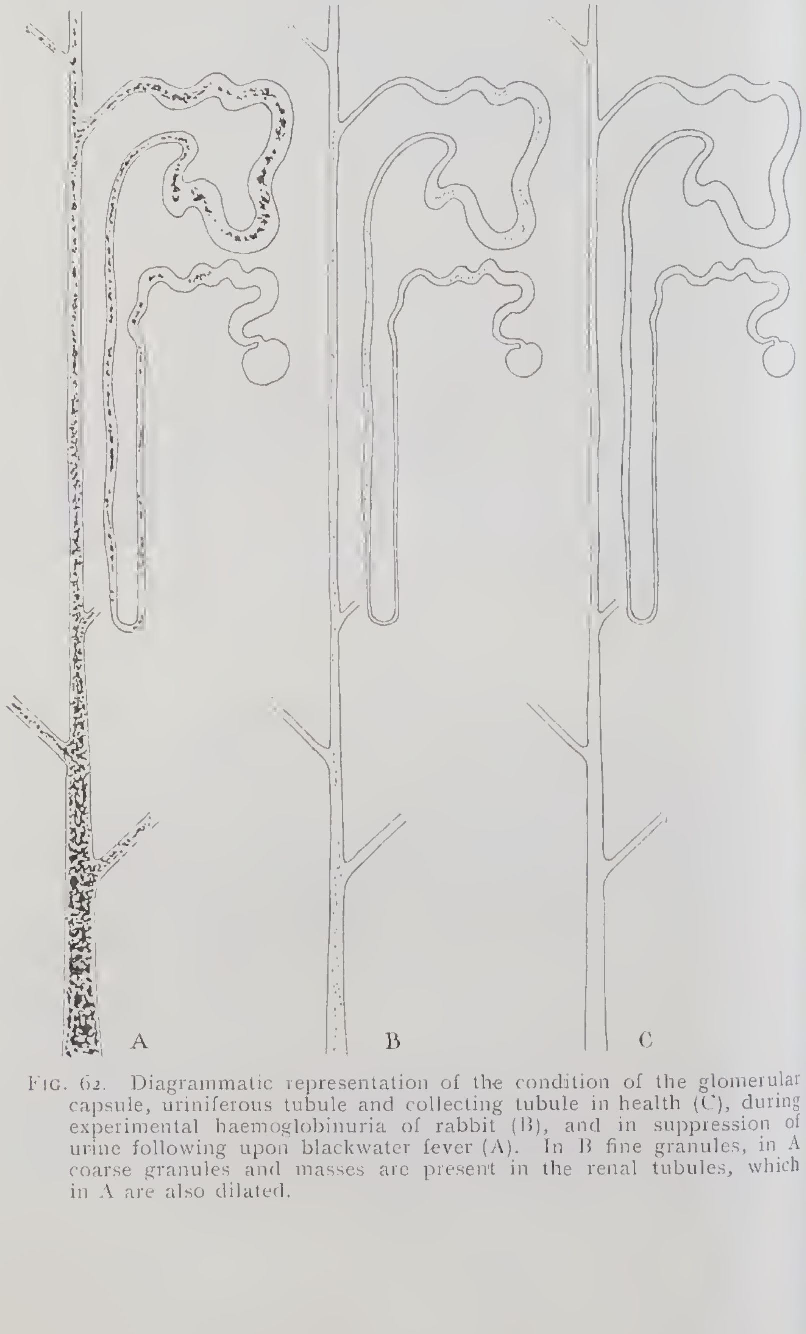

FIG. 62. Diagrammatic representation of the condition of the glomerular capsule, uriniferous tubule and collecting tubule in health (C), during experimental haemoglobinuria of rabbit (B), and in suppression of urine following upon blackwater fever (A). In B fine granules, in A coarse granules and masses are present in the renal tubules, which in A are also dilated.

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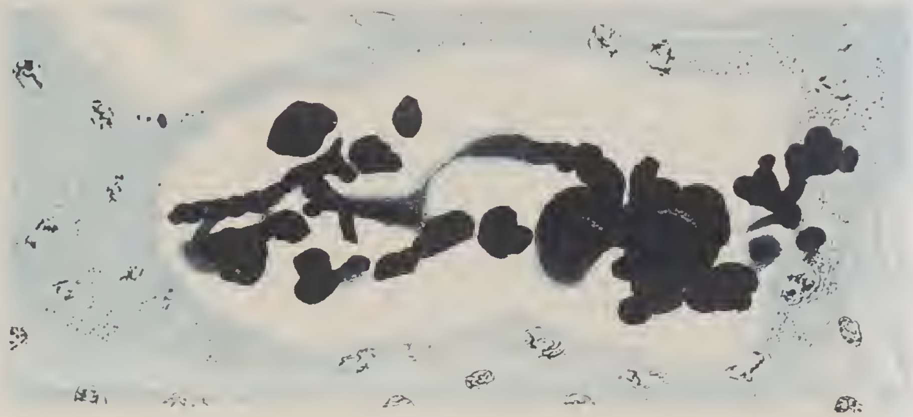

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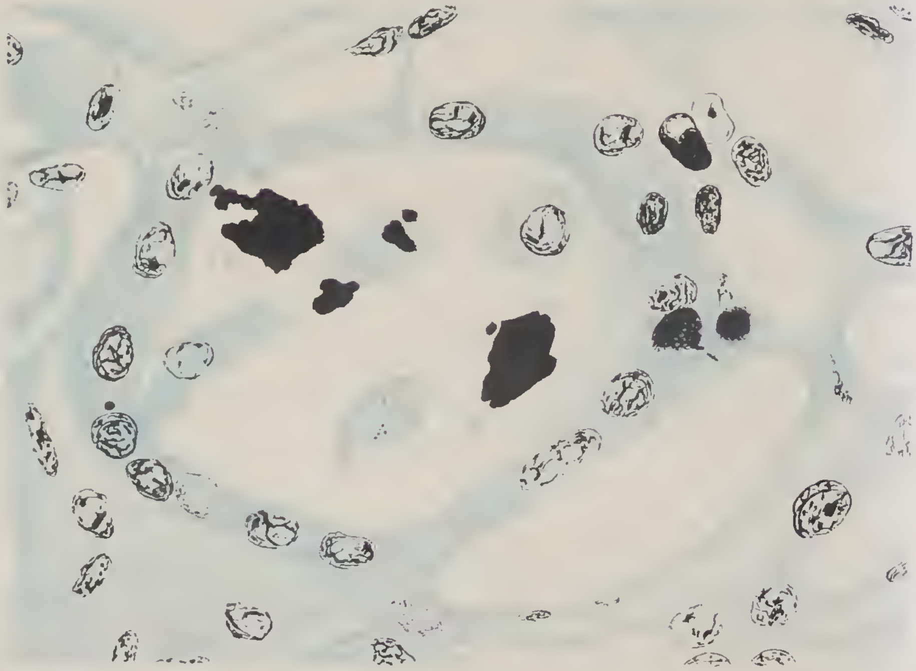

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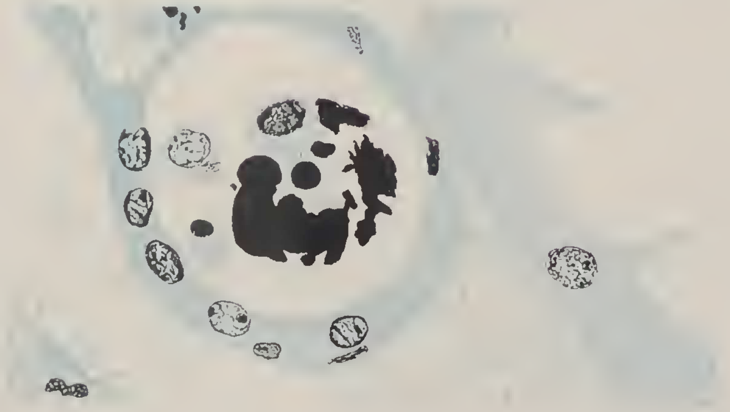

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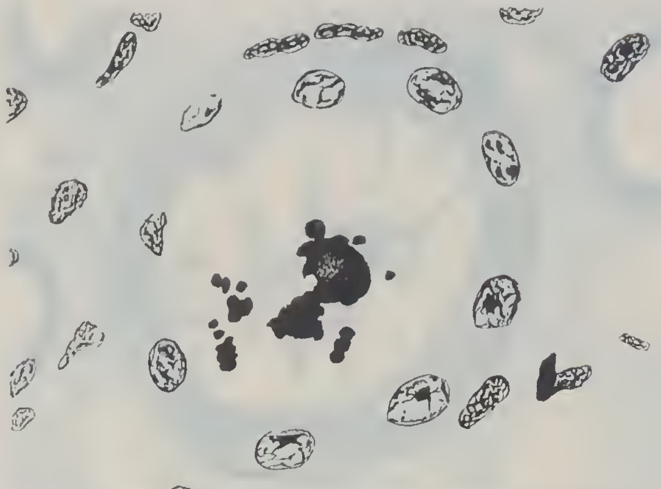

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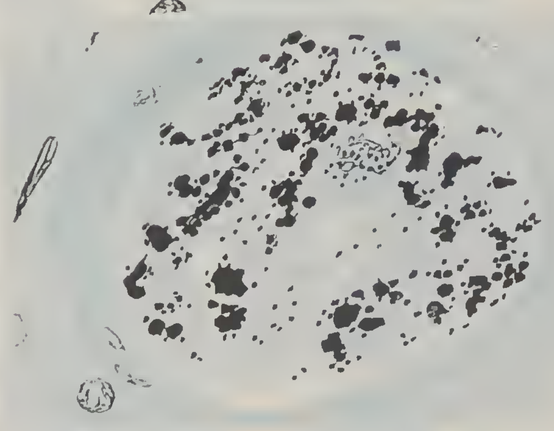

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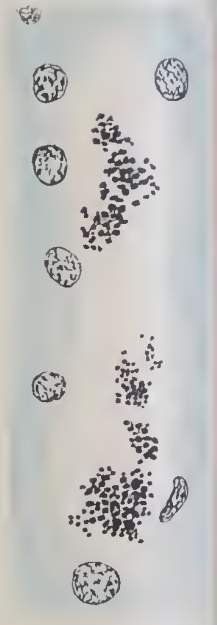

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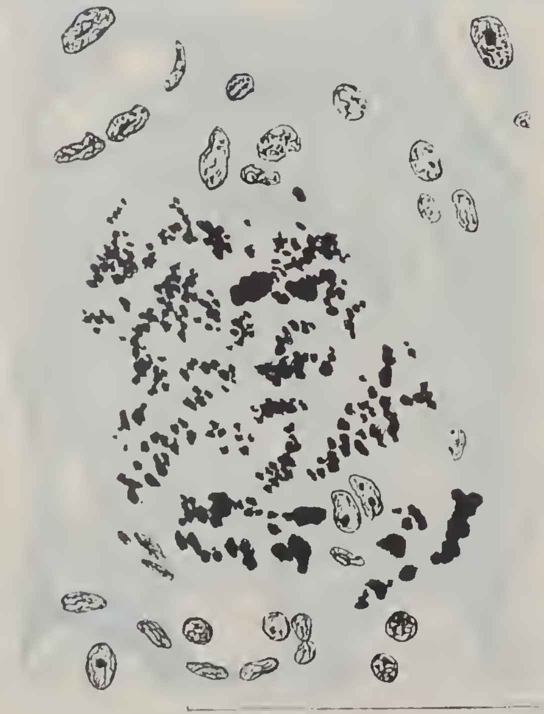

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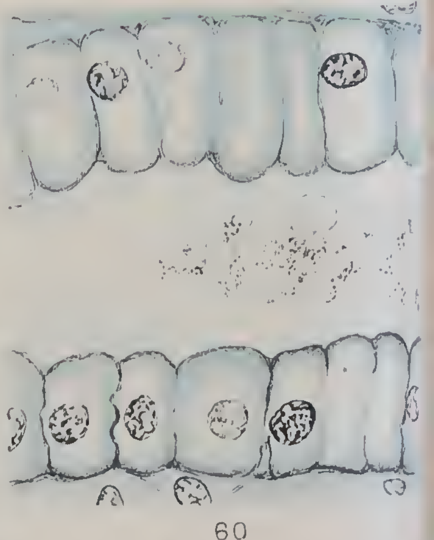

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been observed in blackwater fever, in particular fragmentation of the red cells, were present, and in the kidneys epithelial degeneration was frequently observed. Unfortunately, in consequence of the complexity of these experiments, in which, to use a mathematical expression, several variables occur together, it is difficult to be quite sure of the relation of the pathological conditions present to the haemoglobinuria and anuria, or to be certain of the identity of the anuria so produced with that occurring in blackwater fever, in spite of the similarity of the aspect of the kidneys in the two cases. To ascertain the mechanism of production of the haemoglobinuria and anuria in such investigations it is essential to experiment as far as possible with a single pathological condition at a time. The experiments in question, however, if not as illuminating as could be desired, are very suggestive and of considerable interest.

From the foregoing description of the condition of the kidneys in suppression of urine in blackwater fever, the mechanism of this process becomes at once quite clear. The suppression is not a functional state, but is a purely mechanical process due to blocking of the collecting tubules by plugs composed of densely packed dark reddish granules, identical with the casts observed in the urine during suppression (Cases 7a and 11, Fig. 32), and passing by every transition into the casts ordinarily found during blackwater not attended with suppression, the difference between the two lying in the large size of the granules and greater firmness of the casts in the former as compared with the latter, and also in the firm adhesion to the epithelial lining of the tubules in the former case.

The effect of the plugging is seen in the distension of the uriniferous tubules and Malpighian capsules leading to the considerable general enlargement of the kidney observed at death (Figs. 61 and 62).

DESCRIPTION OF ILLUSTRATIONS

FIG. 53. Section of cortex of kidney during suppression of urine, eight days after haemoglobinuria had ceased. Blackwater Fever, Case 7a. A convoluted tubule is shown across obliquely. The lumen of the tubule, which is dilated, contains numerous darkly stained masses measuring 7μ to 25μ across, consisting of granules, 2μ to 5μ in diameter exhibiting varying degrees of coalescence. The renal epithelium is not markedly changed. No free epithelial cells are seen in the lumen of the tubule. Hardened in alcohol. Stained with iron alum haematoxylin. × 850.

FIG. 54. Section of medulla of kidney during suppression of urine, eight days after haemoglobinuria had ceased. Blackwater Fever, Case 7a. A collecting tubule is shown in transverse section, in the lumen of which are seen darkly stained, two large irregular masses, and several smaller collections of granules. The individual granules measure from 2μ to 4μ in diameter. Four free epithelial cells are also present in the lumen of the tubule. The cytoplasm of the latter is more homogeneous in aspect than that of the epithelial wall of the tubule, which is unchanged in appearance. Hardened in alcohol. Stained with iron alum haematoxylin. × 850.

FIG. 55. Section of cortex of kidney during suppression of urine, five days after haemoglobinuria had ceased. Blackwater Fever, Case 11. An ascending limb of Henle's loop is seen in transverse section. The lumen of the tubule contains darkly stained masses, composed of partially coalesced granules, the smallest of which are about 2μ in diameter. In addition three free renal cells are seen in the tubule. To the right is a portion of a convoluted tubule, and below and to the left a blood vessel. The renal epithelium is very granular in aspect. Hardened in alcohol. Stained with iron alum haematoxylin. × 850.

FIG. 56. Section of medulla of kidney during suppression of urine, five days after haemoglobinuria had ceased. Blackwater Fever, Case 11. A large collecting tubule is shown in transverse section, containing darkly stained granules 1μ to 3μ in diameter, arranged in groups of varying size, and accompanied by lightly stained flocculent and finely granular material. The epithelial wall of the tubule is unaltered in aspect, as is also the interstitial tissue. Hardened in alcohol. Stained with iron alum haematoxylin. × 850.

FIG. 57. Section of cortex of kidney, seven days after haemoglobinuria had ceased. Blackwater Fever, Case 16. A renal tubule, probably an ascending limb of Henle's loop, is shown cut across transversely. The wall of the tubule, which retains its epithelial lining, is faintly stained so that its outlines are indistinct. In the lumen of the tubule are numerous darkly stained granules, varying in size from less than 1μ to about 4μ in diameter, in part arranged in groups. In addition a large amount of lightly tinted granular material is also recognisable. Fixed in Flemming's solution. Stained with iron alum haematoxylin. × 850.

FIG. 58. Section of medulla of kidney, seven days after haemoglobinuria had ceased. Blackwater Fever, Case 16. A collecting tubule, lightly stained, is seen in transverse section. The lumen of the tubule is filled with darkly stained granules, mostly arranged in groups. The individual granules measure 1μ to 3μ in diameter. The epithelial lining of the tubule is partially detached. Unlike the preceding section, there is very little unstained débris contained within the lumen of the tubule. Hardened in alcohol. Stained with iron alum haematoxylin. × 850.

FIG. 59. Section of cortex of kidney during experimental haemoglobinuria. Rabbit 7, Table 35. A renal tubule is shown, containing granules $0{\cdot}5\mu$ to 1μ in diameter, which occupy a relatively small portion of the lumen of the tubule. These granules are darkly stained, but slightly less so than is represented in the Figure. No other débris is present in the tubule. The epithelial lining of the tubule wall is well defined, some of the divisions between the individual epithelial cells being recognisable. Hardened in alcohol. Stained with iron alum haematoxylin. × 850.

FIG. 60. Section of medulla of kidney during experimental haemoglobinuria. Rabbit 7, Table 35. A collecting tubule is shown cut across longitudinally. The lumen of the tubule is for the most part occupied by lightly staining flocculent material containing a few very fine darkly stained granules the diameter of the largest of which does not exceed $0{\cdot}5\mu$. The epithelial lining of the tubule is normal in aspect. Hardened in alcohol. Stained with iron alum haematoxylin. × 850.

The enquiry, which has some bearing upon the prophylaxis of suppression, presents itself; is it necessary, in order that this plugging may occur, that the flow of urine should be diminished? Obviously if the flow were very rapid the tendency to accumulation of granular material in the tubules would be diminished, and with it also the tendency to blocking. The question turns largely upon whether the granular material which is present becomes denser through the absorption of water by the epithelium of the tubules. If this occurs the formation of dense plugs is evidently partly conditioned by the relation subsisting between the rate of flow of glomerular fluid on the one hand and the rate of removal of water from the lumen of the tubules on the other hand. If the latter is sufficiently rapid no retardation of the former may be required in order that plugging may take place.

In view of the mechanical blocking of the kidneys in suppression of urine in blackwater fever, it is astonishing that this process should still be attributed to a purely functional disturbance, a nervous reflex or vasomotor inhibition of secretion of urine, a view which is now chiefly of historical interest. By A. Plehn[*] a nervous inhibition of glomerular secretion is regarded as the primary and essential factor in this condition.

Another point of interest in connection with suppression of urine in blackwater fever is the enquiry whether, when once suppression has been established, the plugs may yet be dislodged and the flow of urine re-established. As a matter of fact such cases occur: four cases are referred to by Werner,[†] in which after two or more days anuria secretion of urine was re-established. In Cases 7a and 11 (pp. 195 and 217) it has already been mentioned that dislodged plugs were constantly present in the scanty urine passed during the period of suppression. We may, therefore, surmise that temporary, and it may be also partial, plugging is in reality a not unfrequent occurrence in blackwater fever and produces for a time a partial or even complete arrest of the flow of urine from a few or from the majority of the renal tubules, such obstruction afterwards passing away, the plugs becoming dislodged by the pressure of urine behind. This condition may be suspected whenever the granular casts found in the urine are large and very firm. It is well known that the amount of urine passed when haemoglobinuria makes its appearance may be scanty. This is illustrated by the following cases, coming under our observation, in which the quantity of urine secreted during the first twenty-four hours of haemoglobinuria was 248 c.cm. in Case 2; 344 c.cm. in Case 3; 170 c.cm. in Case 6a (next day 85 c.cm.); 530 c.cm. in Case 10; and 800 c.cm. in Case 15. So marked a reduction as occurs in the first four cases can only be attributed to renal blocking.

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[*] Ätiologie und Pathogenese des Schwarzwasserfiebers, Virch. Arch., 1903, B. 174, S. 509. On page 519: "das plötzliche Stocken der Urinsekretion stellt aufangs vielleicht ebenfalls einen rein functioneller Vorgang dar, und es folgt erst sekundar die mechanische Verlegung der Harnkanälchen durch Coagulieren der Eiweissüberladenen Flussigkeit." J. de Haan, Die nieren beim Schwarzwasserfieber, Arch. f. Schiffs- und Tropenhygiene, 1905, B. 9, S. 22, explains suppression as in part due to mechanical obstruction by renal plugs, in part due to low glomerular pressure in consequence of which the pressure of the urine is insufficient to drive out the plugs (pp. 30-31). Our observations on experimental haemoglobinuria do not afford any evidence of the occurrence of clotting in the tubules; on the contrary the constituents of the blood which lead to clotting do not pass through the kidney, haemoglobin alone being eliminated.

[†] Loc. cit, p. 14.

The fluid passing into the bladder during suppression obviously consists in part of urine. This is shown by the circumstance that the suppression exhibits in different cases varying degrees of incompleteness, indicating that the blocking of the renal passages is correspondingly incomplete, as is further illustrated by the occasional re-establishment of the flow of urine after suppression of several days' duration. It does not, however, follow that the whole of the fluid passing into the bladder during suppression is urine. The coagulable proteid contained in the urine, which was considerable in amount in Cases 7a and 11, varying from $\frac{1}{4}$ column to $\frac{2}{3}$ column, may enter the renal passages as a result of damage to the renal epithelium consequent upon plugging of the collecting tubules, but a certain amount probably comes also from portions of the tubules in which plugs carrying renal epithelium (Figs. 27, 31 and 32) have become detached and have been discharged into the renal pelvis, so that the basement membrane of the tubules has become exposed. In such cases the lymph contained in the tissue spaces of the kidney would drain into the tubules and mix with the urine. Our investigation, unfortunately, came to an end just as the study of this problem was commenced. It is desirable that further information should be obtained as to the nature of the proteids present, as well as the amount of urea and other nitrogenous substances in the urine during suppression.

SUMMARY

1. During experimental haemoglobinuria dependent upon haemoglobinaemia, as also in the haemoglobinuria of blackwater fever, granular casts of varying size, sometimes very soft, sometimes firm and dense, often containing degenerated nuclei derived from the epithelium of the renal tubules, were met with in the urine (Figs. 21-31).

2. During suppression in blackwater fever (Cases 7a and 11) the urine contained very large firm casts with exceedingly coarse granules, often surrounded externally by epithelium derived from the ducts of Bertini, in which these casts were formed (Fig. 32).

3. The amber-coloured urine passed during suppression contained a large amount of coagulable proteid. The average daily amount of urine passed during nine days of suppression was in one case (7a) 28 c.cm., in another case (11) 66 c.cm.

4. Suppression of urine in blackwater fever is of mechanical origin, due to a blocking of the renal tubules.

V. THE MECHANISM OF PRODUCTION OF BLACKWATER.

The condition of the urine and blood plasma in respect of dissolved haemoglobin in blackwater fever has been studied in Section 3. In the same section also, the relation between haemoglobinaemia and haemoglobinuria has been determined experimentally in rabbits, while the condition of the urine and of the kidneys in suppression of urine in blackwater fever has been described in Section 4. The problem now remaining to be considered is whether the mechanism of production of blackwater is identical with the process induced experimentally in rabbits. Before the consideration of this point can be entered upon it will be necessary to make further reference to the condition of the haemoglobin in the urine in blackwater fever, and in particular to the presence of red cells in the urine, to allude again to the changes undergone by haemoglobin in the urine, and to describe the methods adopted for the estimation in urine of haemoglobin, both when unchanged and when altered by the action of urine.

TABLE 43. Characters of the urine in blackwater fever, in respect of brown coloration, unchanged haemoglobin in solution, and red blood cells.

No. of Case	Colour	Haemoglobin in solution	Red blood cells	Estimation of haemoglobin (maximum amount
1	Porter coloured	Present	Present	—
2	Chocolate coloured	Absent	"	—
3	Porter coloured	Present	"	1·4 % (Fig. 10)
4	Dark red	—	—	—
5	Claret red	Present	—	—
6	Porter coloured	—	Present	—
6a	Brown	Absent	"	0·8 %
7	Porter coloured	"	Absent	0·2 %
7a	"	Present	Absent at first, appeared afterwards	1·2 % (Fig. 11)
8	"	"	Present	0·2 % (Fig. 12)
9	"	—	—	—
10	"	Present	Absent at first, appeared afterwards	2·6 % (Fig. 13)
11	"	"	" "	2·6 % (Fig. 14)
12	Dark red	"	Absent	2·2 % (Fig. 15)
13	(Red)	—	—	—
14	Porter coloured	Present	Absent	3·0 % (Fig. 16)
14a	"	"	"	1·8 % (Fig. 17)
15	"	"	"	3·8 % (Fig. 18)
16	"	"	"	0·8 % (Fig. 19)
17	"	"	Present	3·5 % (Fig. 20)

In Table 43 the condition of the urine in respect of haemoglobin is given in all except three (4, 9, 13) of the cases of blackwater fever coming under our observation. It will be seen (Column 3) that in thirteen out of sixteen cases haemoglobin was present in solution, in the form of oxyhaemoglobin, in amounts varying from 0·2 per cent. to 3·8 per cent., while in addition a further amount of haemoglobin was changed into the brown substance, exhibiting no bands in the solar spectrum, already referred to (p. 51), which gives rise to the porter-colour of the urine. The total amount of dissolved haemoglobin passing into the urine consists of these two fractions, which are determined by the methods given below. Although in three cases (2, 6a, 7) haemoglobin in solution could not be recognised by spectroscopic examination of the brown or porter-coloured urine passed, nevertheless dissolved haemoglobin was in all probability originally present, but had been broken up by the action of the urine (cp. Table 25, p. 53); in Case 2 the urine was retained for several hours before being voided, and micro-organisms were present in the bladder (see clinical notes, p. 178); in Case 6a the percentage of haemoglobin in the urine was 0·8 per cent.; and in Case 7 the percentage of haemoglobin present was also very small, and the urine had stood for some time before being examined. In a subsequent attack (7a) the last patient passed urine containing dissolved haemoglobin.

When the urinary deposit obtained after centrifugalisation was examined (Column 4, Table 43), red blood cells were found to be present in ten out of sixteen attacks, but in three of these (7a, 10, 11) red cells were at first absent from the urine and made their appearance subsequently (clinical notes, pp. 195, 207 and 217). As a rule the red cells were not present in large numbers. They were in fact usually much less numerous than the casts, but an exception occurred in Case 2, in which they were present in very considerable numbers, so as to form at the bottom of the urine glass a brownish red deposit visible to the naked eye. In this case there were a number of decolourised red cells and also red cell stromata. The red cells were sometimes crenated, but beyond this and the partial decolouration just referred to they did not present any other abnormal appearance.

In order to estimate haemoglobin in urine, the following three methods were employed; the first two being employed for the estimation of unaltered dissolved haemoglobin, and the third for the estimation of both unaltered haemoglobin and also haemoglobin which had been broken up by the action of the urine.

Method 1. The urine was centrifugalised and the supernatan liquid diluted with a measured volume of distilled water until the percentage of haemoglobin as judged by the naked eye was abou 0·15 per cent.* The diluted urine was then put into the half cell von Fleischl's haemoglobinometer. If the dilution was found unsuit able a fresh dilution was made until a concentration was obtained which could be easily matched. The haemoglobinometer scale wa previously standardised by means of solutions containing known percentages by volume of healthy human red cells; this having been done, the percentage of haemoglobin in the diluted urine could be a once determined from the scale numbers.

In many cases no difficulty was found in making a determinatio of haemoglobin by this method. Difficulty arose, however, when th haemoglobin had been largely converted into the brown substanc already referred to, a change which is likely to occur when the urin has been retained in the bladder for a long time before being passed or after being voided, has been allowed to stand for some time befor examination. This change is of course relatively more marked i urines containing small, than in urines containing large, percentage of haemoglobin. Another source of difficulty in matching the colou of the diluted urine with that of the tinted glass of the haemoglobino meter occurs when dissolved haemoglobin is discharged in sm amount into urine which is itself of a dark brownish amber colour as not unfrequently happens in malarial patients. Matching als becomes difficult when the urine in the bladder contains bacteria, occurred in Case 2, the turbidity thus caused not being complete removable by centrifugalisation. When from any of these causes becomes impossible to employ the above described method for th estimation of dissolved haemoglobin, recourse may be had to th next method.

Method 2. The urine was diluted, if necessary, with a know volume of distilled water until, in a column one to two centimetr high, the oxyhaemoglobin bands lying between the solar lines D and b were separate and of suitable depth of shade. By the aid of a haemocrit a solution of haemoglobin, containing 0·2 per cent by volume of healthy human red cells, was next prepared. A glass cell one or two centimetres high was then filled with this solution, while the diluted urine was placed in a second cell, the height of which could be varied. The haemoglobin bands of the second cell were then, by varying the height of the column employed, made to match those of the first, for which purpose a Zeiss comparison spectroscope was employed.

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* This rough comparison may be made by putting the centrifugalised urine into a te tube, filling another test-tube with a 0·15 per cent. solution of haemoglobin and compar the two, the former being further diluted or a lesser dilution prepared, as may be necessar With a little practice, however, it becomes possible to obtain the required dilution w sufficient approximation by the naked eye without comparison.

By this method it is possible to estimate percentages of haemoglobin in cases in which colouring matter is present in the urine in amount sufficent to render haemoglobinometer determination difficult or impossible. This method may, of course, be used when the preceding method is also available. The results obtained by the two methods correspond closely. The first method is more rapidly carried out, and is, therefore, when available, preferable to the second.

Method 3. The estimation was made by boiling the solution of haemoglobin. Ten c.cm. of the centrifugalised urine, which did not as a rule require filtering, were rendered fairly acid with acetic acid, boiled and after cooling transferred to a graduated centrifugal tube. Centrifugalisation was continued until the bulk of the precipitate became constant. Its volume was then measured. Previously by means of the haemocrit, a number of specimens of urine (with the addition of 5 per cent. to 25 per cent. of water) were prepared containing known percentages, by volume, of healthy red blood cells (in the moist condition), which were subsequently laked. To these solutions an equal volume of urine was added, and the solutions rendered very slightly acid with acetic acid and then boiled. The bulk of the centrifugalised precipitates was next determined as above described, and from this a table constructed, which permitted the precipitates obtained in haemoglobinuria to be expressed in percentage form. As this method depends upon the precipitation of globulin, and is not affected to any considerable extent by the change undergone by haemoglobin in the urine, it may therefore be employed to determine with fair approximation the total amount of haemoglobin originally present in the urine. The method is, however, only approximate, since the volume of the precipitate varies somewhat with different specimens of urine, and is also affected by the rate of centrifugalisation employed. Table 44 gives the average bulk of precipitate obtained with varying percentages of dissolved haemoglobin in slightly diluted urine. By graduating the centrifugal tube employed in percentages of wet red cells, instead of in cubic centimetres, the former could be read off directly and all calculation avoided. It was found that when an equivalent amount of oxalate blood plasma was added (the red cells require to be separated from the blood plasma for convenience in laking) so that (dissolved haemoglobin and blood plasma were present in their natural relation the bulk of precipitate obtained was increased by 20 per cent. to 2 per cent.

TABLE 44. Bulk of precipitate obtained on boiling 10 c.cm. of slightly acid urine containing varying percentages of dissolved haemoglobin.

Percentage of haemoglobin contained in urine of sp. gr. 1·014 diluted with 5 % to 25 % of water.	Bulk (after centrifugalisation) of precipitate obtained upon boiling after previous slight acidification with acetic acid.
6·5 %	3·00 c.cm.
5·0 %	2·28 c.cm.
4·0 %	1·82 c.cm.
3·0 %	1·36 c.cm.
2·0 %	0·91 c.cm.
1·0 %	0·46 c.cm.
0·5 %	0·23 c.cm.
0·25 %	0·11 c.cm.

By one or the other of these methods the percentage and total amounts of haemoglobin given in Tables 43 and 45 were determined.

In ten attacks of blackwater fever the total amount of haemoglobin passed into the urine was estimated (Table 45) and was found to be represented by between about 1·5 gm. (Case 7) and 75 gm. (Case 14) of wet red cells. In the cases given in Table 45 it will be observed that there is a general correspondence between the percentage and the total amount of haemoglobin, the higher the former the higher the latter. This relation, in the light of the experiments made upon rabbits, given in Table 35, would appear to be merely a coincidence. It will be noted that in the two cases in which suppression of urine occurred (7a and 11) the total amount of haemoglobin appearing in the urine, equivalent to 9 g. of wet re cells in Case 7a and 22 g. in Case 11, was not so high as in Cases 14 and 17, not of unusual severity, in which the total amounts of haemoglobin were equivalent to 75 g. and 32 g. of wet red cells respectively.

TABLE 45. Amount of haemoglobin passing into the urine in blackwater fever.

No. of Case.	Haemoglobin present in urine during attack, expressed in terms of equivalent weight of wet red cells.
3	12 g.
7	1·5 g.
7 a	9 g.
10	8 g.
11	22 g.
14	75 g.
14 a	20 g.
15	19 g.
16	6·5 g.
17	27 g.

The presence of red cells in the urine in blackwater fever suggests the inquiry whether the haemoglobinuria of blackwater fever may not be really dependent upon renal haemorrhage. In the earlier descriptions of cases of blackwater fever the condition of the urine was often described by using the term haematuria, the condition of haemoglobinuria not having at that time become differentiated from that of haematuria. Subsequent writers recognised that the condition present in blackwater fever was haemoglobinuria, and, so far as we know, no recent writer has described haematuria as a sequence of the administration of quinine in malarial subjects. In the haemoglobinuria produced by the administration of potassium chlorate or other poisonous substances, or by the injection of a haemolytic serum (see p. 128), the presence of red blood cells in the urine, in addition to dissolved haemoglobin, is recorded, but in our experiments on rabbits this did not occur. The possibility of blackwater being primarily due to renal haemorrhage was impressed upon us in particular by Case 2 (pp. 178 to 181), in which the red cells were very numerous, while the urine, after centrifugalisation, although porter-coloured, did not exhibit oxyhaemoglobin bands on spectroscopic examination. If renal haemorrhage really occurred in blackwater fever, the red blood cells escaping into the urinary passages would necessarily become laked in all cases in which the specific gravity of the urine was 1·004 or less, for in urine of or below this specif gravity laking occurs almost immediately when red cells are adde If the urine is of the specific gravity 1·006 to 1·008, laking occurs wit more or less slowness, and in urine whose specific gravity is 1·010 upwards, laking does not occur to any considerable extent. If ren haemorrhage were the cause of the haemoglobinuria of blackwate fever the site of the haemorrhage would necessarily be the glomeru of the kidneys, for only here could the blood readily find its way int the urine, while on the other hand marked interstitial haemorrhag would be ordinarily readily discoverable post-mortem, which is no the case. Furthermore, if renal haemorrhage were the cause of th haemoglobinuria of blackwater fever, then the haemoglobinaem observable in this condition would be a secondary consequence of t renal haemorrhage, and would be explicable only on the assumpti that some of the dissolved haemoglobin was taken up by the ren epithelium and passed on into the blood stream.

On the other hand, the experiments made upon rabbits (Table 35 in which the effect of simple haemoglobinaemia, produced with t animal's own haemoglobin, was studied, taken in conjunction wit the circumstance that haemoglobinaemia is present during th haemoglobinuria of blackwater fever, suggests very strongly that this affection the primary condition is haemoglobinaemia.

It remains to be considered, therefore, how far these t alternative hypotheses, namely, primary glomerular haemorrhage an primary haemoglobinaemia, are capable of accounting for t pheuomena of blackwater fever, and, if possible, to decide which the two is concerned in giving origin to blackwater.

The extent to which the theory of a primary haemoglobinaem is in harmony with the various facts already ascertained respect blackwater fever will be first considered, and then the remain hypothesis will be criticised in the same manner. Owing to t importance of this enquiry, it will be dealt with at some length.

1. From the experiments made on rabbits (Table 35) it evident that a primary haemoglobinaemia reaching to as much 0·95 per cent. of haemoglobin, such as occurs in blackwater fe would be attended with the appearance of haemoglobin in the ur Our results do not enable us to say how large the maxim percentage of haemoglobin in the blood plasma in blackwater fe

may be. It is probable, judging from the rapidity with which haemoglobin disappears from the circulating blood in the rabbit, that our observations, which were of necessity few in number, represent less than the maximum percentage. Nor are our observations sufficiently numerous to enable us to state the duration of the haemoglobin, but the observations available in Table 33 and Figs. 13, 18 and 20, suggest that in blackwater fever the discharge of haemoglobin into the blood plasma is a slow process, and the duration of the haemoglobinaemia is therefore much longer than in the experimental haemoglobinaemia of Table 35. The amount of haemoglobin passing into solution in the blood plasma cannot be determined. It would not be possible to make even an approximate calculation of this amount in blackwater fever until the curve representing the degree of haemoglobinuria from beginning to end of the blackwater had been ascertained and the rate at which disappearance of haemoglobin from the living body took place determined. It is, however, easily possible to obtain a figure representing the minimum amount of haemoglobin passing into the blood stream. For example, in an individual whose blood measured 4500 c.cm. and contained 45 per cent. of red cells and 55 per cent. of plasma, if the blood plasma were found on examination during blackwater fever to contain as much haemoglobin as would be present in 1 per cent. of its volume of red blood cells in the moist condition, then the total amount of haemoglobin discharged into the blood plasma would be not less than that contained in 2475 × 0·01 = 24·75 c.cm. of the patient's red cells, or 45 c.cm. of his blood prior to the onset of haemoglobinaemia. The amount actually passing into solution in his blood plasma would be greater than this, for, on the one hand, a single observation of his blood plasma would not reveal the maximum percentage of haemoglobin, and, on the other hand, the whole of the haemoglobin would not be discharged into the blood plasma in the course of a few minutes, as in the experiments recorded in Table 35. Since in the observations recorded in Table 50 the duration of individual attacks of haemoglobinuria ranged from four hours or less to four days, the amount of blood destruction would appear to represent a considerable portion of the total amount of blood present, and would be sufficient to account both for the watery condition of the blood generally observed when the finger is pricked, and also for the marked anaemia which usually attends blackwater fever.

2. The hypothesis of a primary haemoglobinaemia* is in harmony with another characteristic phenomenon of blackwater fever, namely, the appearance of granular casts in the urine. The experimental work on rabbits shows that haemoglobinaemia is necessarily attended with the formation of these casts. The exact mode in which the granular material is formed remains in both cases undetermined. The experiments made by different observers as to the site of elimination of haemoglobin are still regarded as indecisive.† It is uncertain whether dissolved haemoglobin is eliminated by the glomerular or the tubular epithelium: in the former case the granules, which contain iron and are evidently derived from haemoglobin, would be deposited or precipitated in the urine in the tubules and would increase in number, perhaps also in size, as the renal pelvis was approached; in the second case the granules would presumably be formed in the renal epithelium and would then be discharged into the renal tubules, without any subsequent increase in size, though they would become more closely packed if water were removed from the lumen of the tubules by the action of the renal epithelium. Our observations on Cases 7a, 11 and 16 (Figs. 33-44, and 53-58) do not enable us to assert that any increase in the size of the granules takes place as the collecting tubules are approached. In the latter, coarse masses of stainable material were encountered more often than definite granules. Possibly some change in the solid material contained within the tubules occurred during the time elapsing between the cessation of haemoglobinuria and death. In the kidney of the rabbit, in experimental haemoglobinuria (Figs. 45-51 and 59-60), no unmistakable increase in size of the granules could be recognised as the collecting tubes were approached but only a more dense packing of the granules was observable Several authors have, however, described an increase in size of the

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* By this it is not meant to imply that laking necessarily occurs in the peripher circulating blood. What is meant is a haemoglobinaemia not secondary to ren haemorrhage.

† Ponfick, Experimentelle Beiträge zur Lehre von der Transfusion, Virch. Arch., 18 B. 62, S. 273 (cp. Plate), concluded that dissolved haemoglobin was eliminated by the ren glomeruli and red cell fragments by the epithelium of the renal tubules. Marchand, Z Kenntniss der feineren Veränderungen der Nieren bei Hämoglobinausscheidung, Virch Arch., 1883, B. 91, S. 267, who describes a diapedesis of red cells, also adopts this view.

granules towards the distal tubules, both in blackwater fever and in haemoglobinuria due to potassium chlorate and other haemolytic agents. In these cases it may be questioned if the small and large granules in the proximal and distal portions of the renal tubules respectively are of contemporary origin. If the earlier granules, which as secretion of urine proceeds pass along the uriniferous tubules towards the collecting tubules, are large, and those formed later, when haemoglobinuria is passing off, are small, then the latter, by remaining in the upper portions of the tubules, might suggest an apparent increase in size of the granules as they descended towards the renal pelvis.

The presence of epithelial nuclei in the granular casts contained in the renal tubules, and later voided in the urine, supports the hypothesis that haemoglobin is eliminated by the tubular epithelium, which would appear sometimes to suffer a certain amount of degeneration in the process.

In Case 7a (Figs. 29 and 32) the individual granules were of exceptionally large size. Granules nearly as large, but much less densely packed together, were sometimes met with in rabbits during experimental haemoglobinuria (Figs. 22 and 23). Obviously suppression would be most likely to occur when the granules were of large size and numerous, especially if at the same time the amount of water in the urine were diminished in amount as this fluid passed along the uriniferous tubules.

3. The occurrence of a primary haemoglobinaemia would equally well account for the relapses occurring in blackwater fever as for the original attack (cp. Case 10, Table 50). In reference to this, it may be observed that the relapses of short duration, such as occurred in Case 10, are paralleled by the haemoglobinurias of short duration induced experimentally (cp. Exp. 10, Table 35, Fig. 9.)

4. On the other hand the presence of red blood cells in the urine in blackwater fever does not stand in any direct relation to haemoglobinaemia. Putting aside Case 2, which will be referred to separately later, the appearance of red cells appears to be due to the separation from the basement membrane of the epithelial cells of the uriniferous tubules which is observable in cases of suppression and which is indicated by the presence of epithelial casts in the urine in

non-fatal cases of blackwater fever. When granular casts are detached with separation of epithelium (Fig. 32) the possibility of a few red cells passing from the blood capillaries adjacent to the exposed basement membrane is always present and presumably accounts for their occasional presence in small numbers in blackwater fever.

5. In the experiments on rabbits recorded in Table 35 it was observed that the blood plasma after haemoglobinaemia and haemoglobinuria had passed off, was darker in colour than before experiment, though not so dark as in blackwater fever. Nevertheless yellowness of the sclerotics was not observed in experimenta haemoglobinuria in rabbits. It would appear, however, that the orange or brownish amber-colour of the blood plasma was in both cases of similar origin, namely, due to the destruction of haemoglobin with the production of a brownish substance, which remains in solution, just as occurs when haemoglobin is broken up in a test tube under the action of hydrochloric acid or of quinine bihydrochloride

6. The hypothesis of a primary haemoglobinaemia implies a preceding laking of red blood cells. So far, however, the site in which this laking may occur has not been determined (cp. pp. 15 to 160).

7. Haemoglobinaemia is, as such, unattended by pyrexia and general constitutional disturbance. The source of these must therefore be sought in the cause of the haemoglobinaemia.

Turning now to the consideration of the other hypothesis, that of a primary renal haemorrhage the following points require to be taken into consideration.

1. At the onset we are met with a serious difficulty. If the haemoglobinuria of blackwater fever were due to glomerular haemorrhage, the blood escaping being usually, we will suppose, laked at once owing to the low specific gravity of the urine leaving the glomeruli, then to account for this haemoglobinaemia we must assume that the dissolved haemoglobin so formed in the glomerular cavity is subsequently in part absorbed by the epithelium of the uriniferous tubules and then finds its way into the blood plasma. In such a case the amount of dissolved haemoglobin passing into the blood plasma would sometimes be greater than that passing into the urine, for in Case 7a (p. 195), for example, the blood plasma which contained

0·71 per cent. of dissolved haemoglobin would receive more — probably considerably more — than 11 g. of haemoglobin,* while the urine contained about 9 g. of haemoglobin. It is difficult to assume that in such cases so large an absorption of dissolved haemoglobin by the renal epithelium could take place, when it is borne in mind that in experimental haemoglobinaemia (Table 35, Figs. 2 to 9) the stream of haemoglobin is strongly in the opposite direction, even when the percentage of dissolved haemoglobin is considerable in the lumen of the uriniferous tubules and is relatively slight in the blood plasma, as in Exp. 10, Table 35 (Fig. 9). This difficulty appears to be an insuperable one. It must, moreover, be remembered that if haemoglobinaemia were established as a result of glomerular haemorrhage an additional production of haemoglobinuria would thereby result, as in experimental haemoglobinaemia, so that in such a case the elimination of dissolved haemoglobin by the kidney would be a double process.

2. The production of granular casts under this hypothesis would fall into the first of the two possible methods referred to on p. 144, that is to say that we may assume that the granules would be precipitated as the urine containing dissolved haemoglobin passed along the uriniferous tubules. Another explanation might, however, be offered, namely that the red blood cells escaping from the glomeruli were imperfectly laked and that the granules represented red cell débris still remaining. This point is scarcely worthy of serious consideration. It may be observed that the granules seen in the urinary deposit (Fig. 32) and in the sections of the kidney (Figs. 33 36 and 53·4) in Case 7a, and also sometimes in the urinary deposit in experimental haemoglobinaemia (Figs. 22, 23) reach as much as 5 μ in diameter and then present a considerable resemblance to red blood cells. They differ from the latter in their darker colour and smaller size and the absence of the typical form of red cells, though the latter might perhaps be attributed to the circumstance that these granules are densely packed together. Nevertheless we think the last two points and the fact that every transition occurs between the granules seen in Fig. 32 and the smallest granules seen in Figs. 22, 26 and 31, negative the view that the granules in Fig. 32

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* It is assumed that the patient's blood plasma measured 1500 c.c.

represent altered red cells. Another difficulty arises when the attempt is made to explain suppression of urine on the hypothesis of glomerular haemorrhage. Here it becomes necessary to assume that the glomerular haemorrhage is general throughout the kidney, for otherwise a certain number of uriniferous tubules would remain to carry on the work of the kidney. It is easy to imagine that malarial parasites may be present in the glomeruli and damage the capillary wall thus leading to haemorrhage, but the necessity of supposing that this is general throughout the glomeruli of both kidneys and does not occur elsewhere in the blood vessels of the kidneys (for as is pointed out below, interstitial haemorrhage is not met with) seems to be a fatal objection to the hypothesis in question. It may be observed in passing that a high percentage, namely 1·1 per cent. to 2·7 per cent. of haemoglobin in the urine was observed in Cases 7a and 11 in which suppression occurred, but examination of the urine voided at intervals of several hours does not afford a comprehensive idea of what is taking place in the kidney. This will be made clear at once if the varying amounts of urine collected from the bladder at short intervals in Rabbit 10, Table 35 (Fig. 9, p. 84), are considered. It is obvious that only when the urine is collected continuously, and its haemoglobin content determined at short intervals, can a clear idea of the limits of the variation of the rate of elimination of haemoglobin be obtained.

3. As far as the occurrence of relapses is concerned the hypothesis of a glomerular haemorrhage being the source of the haemoglobinuria of blackwater fever must be equally applicable to the relapses and the original attack. This hypothesis if applicable to the original attack should be sufficient to explain also relapses of slight extent, such as those seen in Case 10 (Table 50).

4. The appearance of red blood cells in the urine would be expected as an occasional, if not a frequent, event in glomerular haemorrhage. It might be expected that partially decolourised red cells and red cell stromata would be often met with, and that red cells which were perfectly healthy in aspect would be unusual. In most of the cases (Table 43) in which red cells were found in the urine in blackwater fever, the majority of these cells were normal in aspect.

5. Glomerular haemorrhage would account for the anaemia, sallowness, and watery condition of the blood observed in blackwater fever.

6. Considerable difficulty is, however, experienced when an attempt is made to reconcile the theory of glomerular haemorrhage with the pathological conditions observed in the kidney after death. Lesions in the glomeruli would be expected, and occasionally haemorrhage into the glomerular cavities extending along the uriniferous tubules, while sometimes blood would find its way in considerable quantities into the urine. Moreover, if we suppose the haemorrhage to be due to malarial parasites in the blood capillaries, these parasites would scarcely be likely to be confined to the glomeruli, but would be present in the blood vessels of the kidneys elsewhere and would cause interstitial as well as glomerular haemorrhage. Now the kidneys have been so far free from these pathological conditions. In fact the changes in the kidneys are exceedingly slight and are practically confined to the presence of granular material in the tubules. Other changes, such as degeneration of the renal epithelium are inconstant, and when present should be regarded as complications of blackwater fever or of malaria, and not as essential factors in the production of haemoglobinuria. It may be remarked that superficial haemorrhages were found beneath the capsule of the kidney and in the mucuous membrane of the renal pelvis of Case 7a, but these were evidently of very recent date and must have occurred after the disappearance of the haemoglobinuria. This case cannot therefore be regarded as in any way supporting the hypothesis of glomerular haemorrhage. Case 2 (p. 178) which appears at first sight to give some support to this hypothesis also fails to do so, when examined more critically. As already mentioned red blood cells were present in the urine in considerable numbers in this case, so as to form, on standing, a layer visible to the naked eye. It was found, however, that the red cells were accompanied by a number of pus cells, there being one pus cell to about eight red cells, while the urine, which was neutral in reaction to litmus paper also contained bacilli when voided. When haemoglobin had disappeared from the urine it was found that pus cells and bacilli still remained, and the previous history left no doubt that both had been present before the attack of blackwater fever. It is clear, therefore, that in

this case it could not be asserted that the red blood cells came from the kidneys, but it is more probable, since cystitis was present, that the red cells came from the situation in which pus cells were being discharged. The difficulty of reconciling the hypothesis of glomerular haemorrhage with the pathological findings is so great that from this standpoint alone this hypothesis must be disregarded.

7. As regards pyrexia and associated constitutional disturbance both hypotheses stand on the same level. The symptom of haemoglobinuria, whichever hypothesis is adopted to explain its causation, does not in itself necessarily imply accompanying constitutional disturbance.

The conclusion which follows from the above discussion is that the hypothesis of haemoglobinuria in blackwater fever being due to primary glomerular haemorrhage is untenable; because this theory (1) would not account for the haemoglobinaemia which accompanies blackwater, (2) nor would it furnish a satisfactory explanation of the mechanism of suppression of urine, while (3) it is out of harmony with the pathological findings in cases of blackwater fever in which death has occurred on the day of appearance of haemoglobinuria. On the other hand the hypothesis of a primary haemoglobinaemia furnishes a satisfactory explanation not only of the pathological findings, but also of the main symptoms of blackwater fever, namely the appearance of haemoglobinuria and of granular casts in the urine, which the experiments on rabbits, recorded in Table 35, prove to be a necessary consequence of haemoglobinaemia in such degrees as were observed in blackwater fever cases (Table 34, p. 75). In the light of all these facts it may, therefore, be confidently asserted that the haemoglobinuria of blackwater fever is dependent primarily upon haemoglobinaemia and is not due to renal haemorrhage. The site of the laking of red cells, causing haemoglobinaemia, remains to be ascertained. The mechanism of the rise of temperature and accompanying symptoms is also left unexplained. The former problem will now be considered; the latter is not capable of elucidation in the present state of our knowledge.

Concerning the site in which laking takes place, there are a limited number of data available. Such laking may occur in the blood in the peripheral circulation or in internal organs.

Now it is well known that malarial parasites may be found in red blood cells before the onset of, and during, blackwater, and it is stated by Stephens* that, either on the first day of blackwater or on

TABLE 46. Examination of blood films in cases of blackwater fever. Leishman's stain.

Case	Time	Malarial parasites	Pigmented leucocytes
1	2nd day	None found	None found
,,	3rd day	,,	,,
,,	4th day	,,	,,
,,	7th day	,,	,,
,,	15th day	,,	,,
2	1st day	,,	,,
,,	2nd day at 9.0 a.m.	,,	,,
,,	2nd day at 2.15 p.m.	,,	,,
,,	3rd day at 2.30 p.m.	,,	,,
3	5th day at 3.30 p.m.	Very scanty	,,
,,	6th day at 6.0 p.m.	None found	,,
,,	6th day at 10.0 p.m.	,,	,,
,,	7th day at 11.45 p.m.	,,	Very scanty
,,	8th day at 10.0 a.m.	,,	None found
,,	15th day	,,	,,
4	5th day at 10.0 a.m.	,,	,,
,,	6th day	,,	,,
,,	16th day at 9.30 a.m.	,,	,,
5	5th day at 1.0 p.m.	,,	,,
,,	6th day at 10.0 a.m.	,,	,,
6	3rd day at 4.0 p.m.	,,	,,
6a	3rd day at 4.30 p.m.	,,	,,
7	4th day at 12.30 p.m.	—	—
,,	7th day	None found	None found
7a	1st day at 10.0 a.m.	,,	,,
8	5th day at 1.45 p.m.	,,	Very scanty
,,	6th day at 10.0 a.m.	,,	None found
9	8th day at 10.0 a.m.	,,	,,
10	2nd day at 11.0 a.m.	,,	,,
,,	3rd day at 10.30 a.m.	,,	,,
,,	5th day at 11.0 a.m.	,,	,,
,,	6th day at 11.0 p.m.	,,	,,
11	3rd day at 7.0 p.m.	,,	,,
,,	4th day at 10.0 a.m.	,,	,,
,,	7th day at 10.30 a.m.	,,	,,
12	2nd day at 4.30 p.m.	,,	,,
,,	3rd day at 10.30 a.m.	,,	,,
13	—	—	—
14	4th day at 5.30 p.m.	None found	None found
,,	5th day at 10.45 a.m.	,,	,,
14a	3rd day at 12.30 p.m.	,,	,,
15	1st day at 9.30 p.m.	,,	,,
,,	2nd day at 10.0 p.m.	,,	,,
16	3rd day at 10.0 a.m.	,,	,,
17	1st day at 11.30 p.m.	,,	,,
,,	2nd day at 9.0 a.m.	,,	,,
,,	2nd day at 3.15 p.m.	,,	,,
,,	3rd day at 10.30 a.m.	,,	,,
,,	4th day at 11.45 a.m.	,,	,,

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* A System of Medicine, Clifford Allbutt and Rolleston, article Blackwater fever, London, 1907, vol. II, pt. 2, p. 293.

the day preceding the attack, malarial parasites have been shown to be present in the majority of cases, that is in 62 per cent. to 96 per cent. of the attacks investigated, but it is also well known that the actual percentage of red cells, which can be observed to be affected, is frequently very small. If, however, the haemoglobinaemia met with in blackwater fever were due to the haemolysis of red cells affected with malarial parasites, present in the peripheral blood, then the affected red cells would be in such numbers as to be easily recognised. For example, a condition of haemoglobinaemia in which the blood plasma contained 1 per cent. of dissolved haemoglobin (cp. Obs. 38, Table 34, for example) would necessitate a minimum affection of one red cell in every hundred, while if this degree of haemoglobinaemia were prolonged for several hours the proportion of red cells affected would be considerably greater. In the cases of blackwater fever coming under our observation (Table 46) parasites* were found in the peripheral blood on the day before the appearance of blackwater in one or two cases; on the first day of blackwater in not one out of nineteen observations (ten attacks); and in no observation made during blackwater fever at a later date. Observations of this kind are, however, inconclusive (and the same objection applies to spleen smears about to be described), for the condition observed cannot safely be assumed to represent that present at the onset of the attack. What is required, in investigating the situation in which laking may occur, is a quantitative examination of the distribution of malarial parasites in the red cells made just before haemoglobinaemia commences. In those attacks in which haemoglobinuria is the direct sequence of a single dose of quinine the time at which haemoglobinaemia commences can be determined. It is, however, only in exceptional cases that observations are likely to be made at this time, since in the majority of attacks the first indication of blackwater fever is the condition of the urine. It follows, therefore, that the data necessary to determine the relation of blackwater fever to malarial parasites contained in the peripheral blood will be obtained with considerable slowness. Until these data are obtained it will not be possible to make any definite statement as to the dependence of blackwater upon haemolysis, in the peripheral blood, of red cell

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[*] About five thousand red cells were examined in each film.

containing malarial parasites. On one occasion (Case 2, Table 46) it happened that an examination of the blood was made within two hours of the establishment of haemoglobinuria and one hour before the administration of quinine. In this case no parasites could be recognised after careful search. It can be asserted, therefore, that in this case haemoglobinuria was not dependent upon the haemolysis, in the peripheral blood, of red cells containing malarial parasites. It is not, however, permissible to draw a general conclusion, applicable to all cases of blackwater fever, from this single instance. In passing it may be observed that the assumption, which is frequently encountered in the literature of blackwater fever, that considerable (that is to say, up to 20 per cent. or more) destruction of red cells occurs in the peripheral blood during an attack of blackwater fever, an assumption based upon red cell counts per cubic millimetre made before and after the attack, has not yet been established, because the total volume of the blood has not been determined in these cases and the influence of any accompanying variation in the volume of blood plasma thereby determined, nor has the high degree of haemoglobinaemia which would be expected to result from such extensive red cell destruction been met with in our observations. It may be added that in the blood films referred to in Table 46, made during and immediately before haemoglobinuria, the red cells were natural in aspect, no partially decolourised cells or stromata being seen.

If, instead of the peripheral blood, the blood in internal organs is considered, data of two kinds are available, the one relating to the enlargement of the spleen in blackwater fever, the other relating to the presence of malarial pigment or, less frequently, malarial parasites in the blood vessels of internal organs. Splenic enlargement is a common feature in attacks of blackwater fever. In the twenty attacks coming under our notice the condition of the spleen is given in Table 47. This organ was found not to be appreciably enlarged in one case (7) in which the attack of blackwater fever was slight, though two months later its lower border projected one and a quarter inches below the costal margin; in four attacks the condition of the spleen was not recorded (6a, 13, 14a, 15), but in two of these cases it was observed to be enlarged two months earlier (6a, 14a); in the remaining fifteen attacks the spleen was enlarged, slightly in four attacks and markedly, that is to say projecting one to four inches below the costal margin, in the remaining eleven attacks. The illne was severe in those attacks in which the enlargement of the sple was greatest; in those in which the enlargement was slight or n appreciable (7, 12, 14, 16, 17) the attack may be described as mil except in Case 16 (p. 235), in which death occurred seven days aft the haemoglobinuria had ceased.

TABLE 47. Condition of spleen in blackwater fever.

Case	Spleen
1	Lower border projects 1½ in. below costal margin.
2	,, ,, 3½ in. ,, ,,
3	,, ,, 2 in. ,, ,,
4	,, ,, 1½ in. ,, ,,
5	,, ,, 1 in. ,, ,,
6	Enlarged and palpable.
6a	(Two months later) condition could not be satisfactorily determined
7	Not appreciably enlarged.
7a	(Two months later) lower border projects 1¼ in. below costal margin.
8	Enlarged and easily palpable.
9	Lower border projects 4 in. below costal margin.
10	,, ,, 1½ in. ,, ,,
11	Enlarged and easily palpable.
12	Slightly enlarged, just palpable.
13	Not examined.
14	Just palpable.
14a	(Three and a half months later) condition not recorded.
15	Condition not recorded.
16	Slightly enlarged.
17	,, ,, just palpable.

The parenchyma of the spleen was examined by means of splen puncture in six attacks (Table 48). This observation is readi carried out. The patient should be instructed to take a moderat inspiration and then hold his breath. A needle, about one millimetre in diameter, attached to a glass syringe, is then passed quickly through the abdominal wall into the substance of the enlarged spleen The piston of the syringe is then drawn out a short distance, after which the barrel is detached, and the needle at once withdrawn From the small amount of parenchyma in the point of the needle films are then prepared, and stained by Leishman's method. Care should be taken not to change the direction of the needle at any time during this procedure. After a little practice puncture can be carried out in about five seconds. Two of the smears obtained in Table 4 were made during haemoglobinuria (Obs. 4 and 5), and the remaining four cases at the close of haemoglobinuria or two to seven days later. In all cases the results were the same (Figs. 63–68). No malarial parasites were found, nor was malarial pigment observed. The red

TABLE 48. Condition of parenchyma of spleen in blackwater fever and malaria (Cp. Table 4).

No. of Observation	Illness	Spleen smear	Period at which examination was made	Relation to haemoglobinuria
1	Suppression of urine after blackwater, Case 7a	Phagocytosis of red cells (by less than 1 % of the white cells). No malarial parasites nor pigment (Fig. 63).	On 9th day, [illegible] hrs. after death. (Cp. Fig. 11.)	Seven days after haemoglobinuria had ceased.
2	Suppression of urine after blackwater, Case 11	Phagocytosis of red cells (by less than 0·1 % of the white cells). No malarial parasites nor pigment (Fig. 64).	On 6th day. (Cp. Fig. 14.)	Two days after haemoglobinuria had ceased.
3	Blackwater fever, Case 12	Phagocytosis of red cells (by 3 % of the white cells). No malarial parasites nor pigment (Fig. 65).	On 2nd day. (Cp. Fig. 15.)	At close of haemoglobinuria.
3 A	Blackwater fever, Case 12	Phagocytosis of red cells (by 3 % of the white cells). No malarial parasites nor pigment.	On 3rd day. (Cp. Fig. 15.)	Twenty-four hours after haemoglobinuria had ceased.
4	Blackwater fever, Case 14	Phagocytosis of red cells (by less than 1 % of the white cells). No malarial parasites nor pigment (Fig. 66).	On 4th day. (Cp. Fig. 16.)	During haemoglobinuria.
5	Blackwater fever, Case 15	Phagocytosis of red cells (by less than 1 % of the white cells). No malarial parasites nor pigment (Fig. 67).	On 1st day. (Cp. Fig. 18.)	During haemoglobinuria.
6	Blackwater fever, Case 16	Phagocytosis of red cells (by about 1 % of the white cells). No malarial parasites nor pigment (Fig. 68).	On 3rd day. (Cp. Fig. 19.)	Towards close of haemoglobinuria.
7	Malaria	Phagocytosis of red cells (by about 0·1 % of the white cells). No malarial parasites nor pigment (Fig. 69).	In morning, T. normal (T. 103° F. on same and preceding evenings.)	—
8	Malaria	No phagocytosis of red cells. No malarial parasites nor pigment.	In morning, T. normal (T. 102° F. in evening.)	—

cells were normal in aspect. The only change recognisable was phagocytosis of red cells on the part of the mononuclear leucocytes. This condition, to which attention was directed by Christophers and Bentley,* was found to be slight in degree, the number of leucocytes

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* Note on the phagocytosis of red blood corpuscles in the spleen of a case of Blackwater Fever, Indian Medical Gazette, 1908, p. 81; Blackwater Fever, Scientific Memoirs by Officers of the Medical and Scientific Departments of the Government of India, No. 35, Simla, 1908, p. 51.

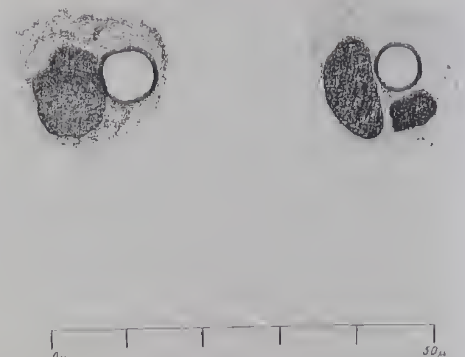

FIG. 63. Spleen smear, Blackwater Fever, Case 7a. Made at autop seven days after cessation of haemoglobinuria (succeeded by suppre sion of urine). To the left is seen a mononuclear leucocyte containing a red cell, which retains its haemoglobin unaltered. To the right a cell with two nuclei and an ingested red cell, whose haemoglobin unaltered. Leishman's stain. × 1,000.

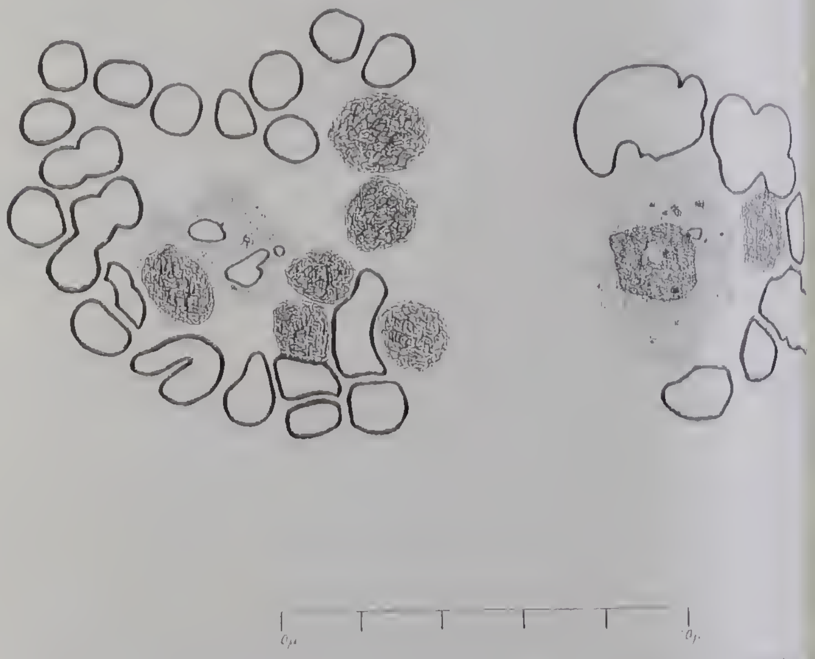

FIG. 64. Spleen smear, Blackwater Fever, Case 11. Made two day afte cessation of haemoglobinuria (succeeded by suppression of urine). T the left a large mononuclear cell with abundant cytoplasm in which are contained three fragments of red cells retaining their haemoglobin five lymphocytes are seen, as well as numerous free red cells. To the right a mononuclear cell with abundant cytoplasm, in which is see a single red cell fragment, the haemoglobin of which is preserve still further to the right a lymphocyte; around are red cell Leishman's stain. × 1,000.

containing red cells being usually considerably less than 1 per cent of the whole number present (Table 18). It is not confined to blackwater fever. It was met with in spleen smears in one out of two cases of malaria (fig. 69) and in the circulating blood of a calf affected with piroplasmosis (fig. 70).

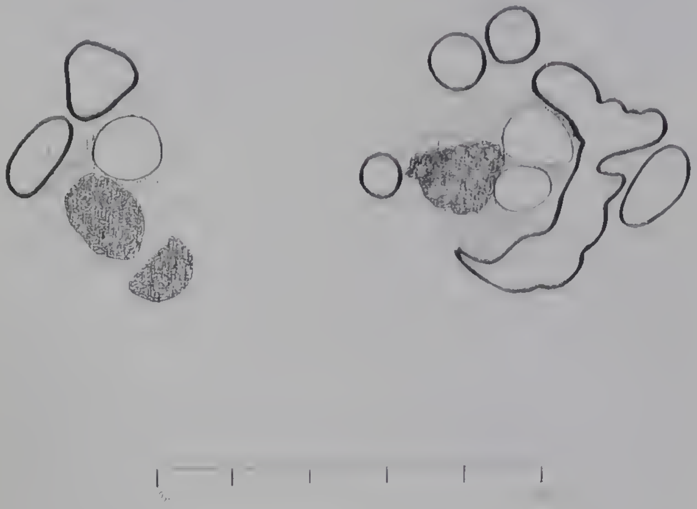

Fig. 68.—Spleen smear. Blackwater fever. Case 12. Made at the close of haemoglobinuria. To the left is seen a mononucleated cell in the cytoplasm of which is contained a partly decolourised red cell; above and to the left are two red cells, below and to the right is a lymphocyte. To the right is shown a large mononucleated cell, the cytoplasm of which contains two decolourised red cells, around are red cells, single and grouped. Leishman's stain. × 1,000.

The presence of malarial pigment in the blood vessels of the organs, usually the kidneys, has been described by several authors. In the three cases in which we had an opportunity of studying this point (Cases 7a, 11 and 10) no pigment was found in the kidneys, spleen or liver, nor could malarial parasites be recognised in the blood vessels of these organs.

By way of summing up the above observations, it may be said that there are not at present sufficient data available to indicate where laking of red cells, causing haemoglobinaemia and thus leading to haemoglobinuria, takes place in blackwater fever.

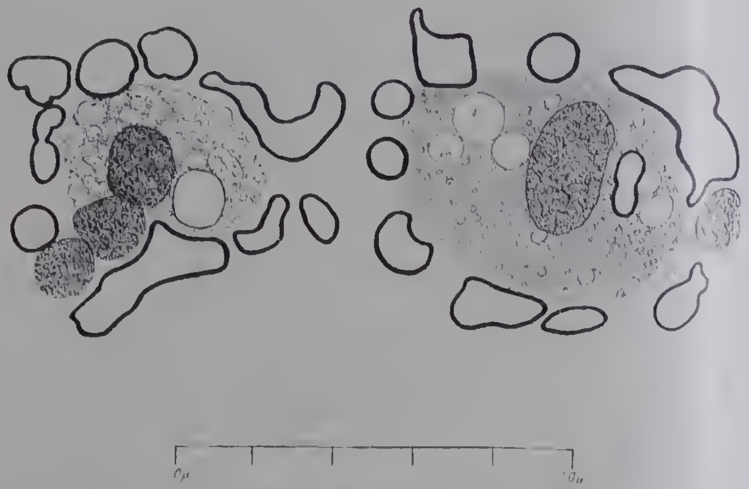

FIG. 66. Spleen smear, Blackwater Fever, Case 14. Made during haemoglobinaemia. To the left three mononuclear cells, one of which has abundant cytoplasm of a reticular character, containing a partly decolourised red cell: around are red cells, single or adherent in groups. To the right is seen a large mononuclear leucocyte surrounded by red cells; in the cytoplasm of the former is seen towards the right a red cell retaining its haemoglobin; immediately adjoining the nucleus on the left is a vacuole, the contents of which are stained slightly red, representing apparently the remains of an ingested red cell; in addition there are present in the cytoplasm numerous colourless vacuoles of varying size, usually with clear contents. Leishman's stain. × 1,000.

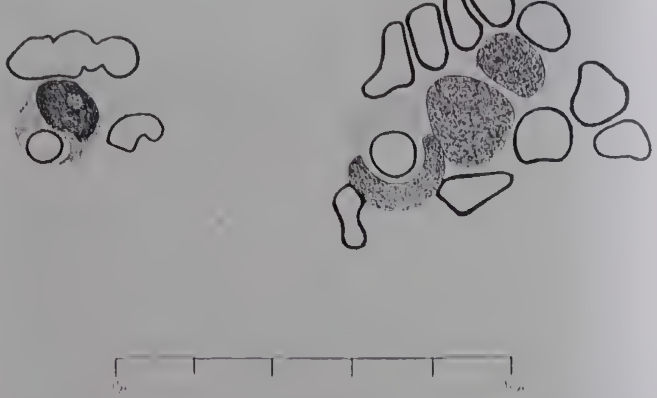

FIG. 67. Spleen smear, Blackwater Fever, Case 15. Made during haemoglobinuria. To the left is seen a mononuclear lymphocyte, in the cytoplasm of which is a red cell which retains its haemoglobin and is surrounded by a clear space; above and to the right are red cell masses. To the right are seen three nuclei, one of which has abundant cytoplasm, in which is contained a red cell whose haemoglobin is preserved unchanged; numerous free red cells are seen around. Leishman's stain. × 1,000.

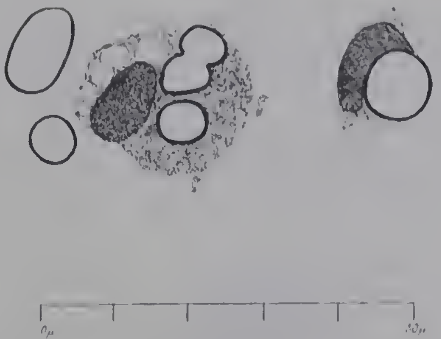

FIG. 68. Spleen smear, Blackwater Fever, Case 10. Made toward end of haemoglobinuria. To the left is a mononuclear cell with abundant cytoplasm, in which are three red cells with unchanged haemoglobin, two being in contact and the third separate; five vacuoles are seen in the cytoplasm; to the left are free red cells. To the right is a small mononuclear cell with scanty cytoplasm, containing a red cell, the haemoglobin of which is preserved unchanged. Leishman's stain. × 1,000.

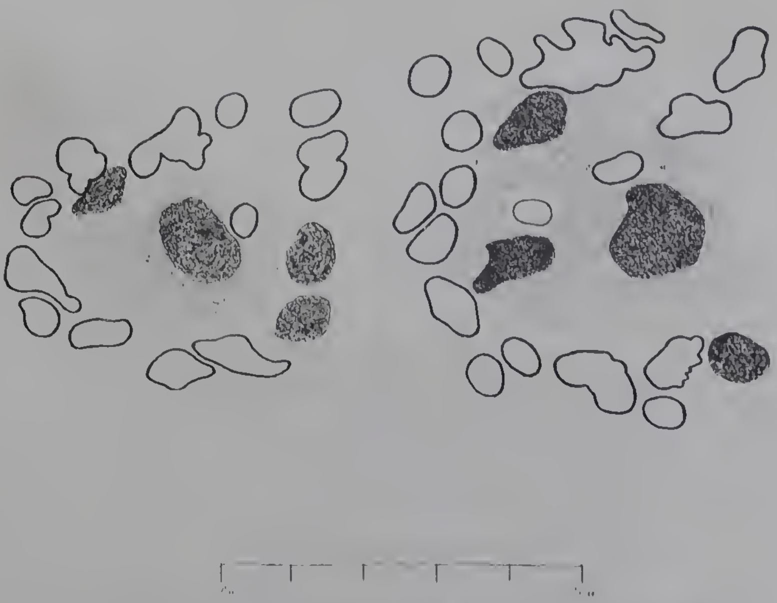

FIG. 69. Spleen smear, Malaria. To the left a large mononuclear cell with abundant cytoplasm containing a red cell with unaltered haemoglobin; around are three lymphocytes and numerous red cells. To the right are seen three nuclei, the cytoplasm of which has coalesced to form a single mass in which are two red cells, one of which (to the right) retains its haemoglobin, while the other, contained in a vacuole, is partly decolourised; around are numerous red cells, mostly in groups; below and to the left is a lymphocyte. Leishman's stain. × 1,000.

Pyrexia and associated symptoms, consisting of rigor, sweatin loss of appetite, vomiting, malaise, headache, cramps in the legs, pa in the back, cardiac weakness, collapse, pain on micturition, and rap anaemia, were met with in varying degree in the cases coming unde our observation, as the clinical records show (pp. 170 to 256). Genera there was severe constitutional disturbance, especially at the onset

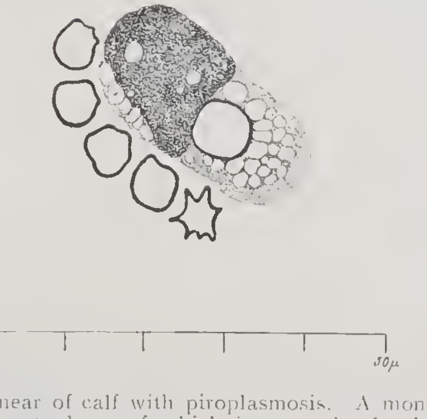

FIG. 70. Blood smear of calf with piroplasmosis. A mononuclear cell is shown, in the cytoplasm of which is a partly decolourised red cell together with numerous small vacuoles. Five free red cells are seen. Leishman's stain. × 1,000.

haemoglobinuria, but subsequently, when haemoglobinuria had cease these symptoms gradually subsided. In sixteen out of the twent observations recorded in Table 49 it will be noted that the attack blackwater was ushered in by a rise of temperature usually reachin 103° F. to 105° F. and commencing with a rigor. The symptom during the attack, regarded apart from the haemoglobinuria, wer similar to those of malaria. Upon recovery more or less weakne anaemia and loss of flesh still remained.

A striking feature of the attacks was that in sixteen of the twent attacks investigated the onset was preceded for several days by mo or less marked general disturbance. This lasted in fourteen attack from one to seven days, and in two attacks ten and twenty-one day respectively, as will be seen on reference to Table 49. Th preliminary illness was regarded as malarial, but in only one cas (Case 3) were malarial parasites found before the onset of haem globinuria (they were sought for on the day before the onset Cases 2 and 3).

[illegible] of constitutional disturbance during blackwater fever, and of preceding illness when present (Cp. with Table [illegible], p. [illegible])

No. of Attack	Malaria	Illness preceding attack of blackwater fever	Blackwater fever
1	Suffered much from malaria. Last attack one month ago.	No illness during fortnight before attack of blackwater fever.	Commenced with rigor, followed by haemoglobinuria. On second day vomiting, rise of temperature to [illegible] in evening. On third day subsidence of symptoms.
2	Has had several attacks of malaria during last eight years. Last attack many months ago.	Slight malaise for a week. On the day preceding blackwater suffered from pyrexia, vomiting, diarrhoea, and restlessness.	Commenced with rigor, followed by profuse perspiration, attended with blackwater: temperature [illegible] F. Next day improvement. On following day constitutional disturbance disappeared.
3	Has been almost free from malaria for last five years.	Malarial rigor five days ago, repeated three days ago, again two days ago, and also yesterday.	Commenced with shivering and vomiting, temperature [illegible]4°, attended with haemoglobinuria. Next day vomiting troublesome, temperature [illegible]2°. Better on following day. Symptoms then subsided.
4	Has suffered much from malaria during last seven years. Last attack about a year ago.	None. On thirteenth day again felt unwell, had pain in back, and vomited.	Commenced with headache, temperature [illegible] F., followed by haemoglobinuria. Still vomiting on second day. Headache on third day. Symptoms subsided by fifth day. On fourteenth day had a rigor, followed by haemoglobinuria, temperature [illegible] F. Next day symptoms subsided.
5	States that he has not had malaria.	Two days before appearance of haemoglobinuria had a rigor, followed by profuse sweating. Anorexia, but no vomiting.	Commenced with rigor and severe constitutional disturbance, attended with haemoglobinuria. Next day symptoms commenced to subside.
6	Has had much malaria during last fourteen years. Date of last attack uncertain.	Felt unwell for last four days, but continued at work until yesterday. Regarded his illness as due to malaria.	Commenced with severe constitutional disturbance, accompanied with haemoglobinuria. The latter disappeared on the third day. Vomiting was troublesome on third and fourth days. Symptoms subsided on [illegible] day.
[illegible]	Last attack of malaria three weeks ago.	Felt unwell the day before the attack of blackwater fever.	Commenced to feel ill, temperature rose to [illegible] F., passed blackwater, vomited severely. Symptoms [illegible] next three days, then subsided on the fifth day.
7	Last attack of malaria six months ago. Has had two previous attacks of malaria.	Felt ill three days ago, went to bed in afternoon. Next day felt unwell, but went to work, then had an attack of ague attended with shivering and profuse sweating. On the following day felt well in the morning, then vomited, and temperature rose to [illegible]4° F.	Passed blackwater, had pain in back and [illegible], but otherwise no symptoms, temperature normal. Next day [illegible]
[illegible]	No attacks of malaria [illegible]	Few days ago felt unwell. Next day had a rigor, followed by profuse sweating, patient's temperature rising to [illegible]4° F. This illness, which was attributed to malaria, [illegible] the temperature remaining raised.	Rise of temperature to [illegible] F., accompanied with haemoglobinuria, profuse sweating, and severe constitutional disturbance. Next day [illegible]
[illegible]	States that he [illegible]. Date of last attack [illegible].	Felt [illegible] for last three days.	Had a rigor, temperature rose to [illegible]4 F. [illegible]

TABLE 49—continued

No. of Attack	Malaria	Illness preceding attack of blackwater fever	Blackwater fever
9	States that he has had malaria many times. Date of last attack uncertain.	Has been unwell for last three weeks, attributing his illness to malaria.	Has a rigor attended with blackwater and temperature of 104·2. Next day vomiting troublesome. Haemoglobinuria ceased in fourth day. Constitutional symptoms subsided on fifth day.
10	During last two years has had much fever. Last attack three weeks ago.	Three days ago suffered from sore throat. On the following day temperature rose to 102° F.	Commenced with rigor, temperature rising to 105° F., with accompanying haemoglobinuria and vomiting. Haemoglobinuria continued with numerous remissions for fifteen days. Cp. Table 51, Fig. 13, and Clinical Notes p. 207.
11	Has had much fever during last nine months.	Has been unwell for a week; was much worse the day before blackwater appeared.	Felt very ill, temperature rose to 103° F., haemoglobinuria appeared, was very restless, vomited. On the third day suppression of urine occurred.
12	During the last six months has suffered from malaria two or three times a month.	Commenced to feel unwell the day before blackwater appeared.	Commenced with a rigor, followed by haemoglobinuria. The latter, together with general symptoms had completely subsided on the third day.
13	From time to time had malaria. Date of last attack uncertain.	None.	Illness commenced suddenly with vomiting and rise of temperature to 104° F., attended with blackwater. Then hyperpyrexia and death on same day.
14	During last five years has suffered considerably from malaria. Date of last attack uncertain.	Has been feeling unwell of late. Became ill with temperature of 100° F. and severe headache the day before the onset of blackwater.	Commenced with severe rigor and vomiting, temperature rising to 105·4° F., with accompanying haemoglobinuria. Next day another severe rigor, temperature reaching 106° F. On the following day condition improved, and symptoms had subsided two days later.
14a	Date of last attack uncertain.	None.	Commenced with rigor and vomiting, temperature rising to 101·4°; then haemoglobinuria appeared. On the second day vomiting continued. On the third day had a severe rigor and temperature of 102° F., vomiting and insomnia continuing. On the fourth day all symptoms ceased.
15	During last six months has suffered much from malaria. Date of last attack uncertain.	Has been unwell for last four days, troubled with anorexia and insomnia, and unable to work.	Haemoglobinuria appeared without rigor; no marked constitutional disturbance at first, but in evening slight vomiting and temperature of 103·6° F. On the second and third days temperature still raised. On the fourth day general condition much improved. On the fifth all symptoms ceased.
16	During last four years has suffered much from malaria. Date of last attack uncertain.	Has been unwell for last four days.	Passed blackwater this morning; was obviously very ill, temperature 101·6° F.; has severe vomiting and diarrhoea. Haemoglobinuria ceased on third day, but albuminuria appeared, and patient continued in a very weak condition till death on the tenth day.
	For many years has had	Was taken ill two days ago, temperature rising to	Illness continued, haemoglobinuria appeared, temperature

One of the patients suffering from blackwater fever (Case 5) denied that he had ever had malaria; he had, however, an enlarged spleen, the lower border of which projected about one inch below the costal margin. With this exception all the patients coming under observation with blackwater fever had in the past suffered from repeated malarial attacks (Table 49, Col. 2). Although the diagnosis appeared to have been in all cases purely clinical, there is no reason to doubt its accuracy (cp. Table 47).

In attempting to interpret the significance of the preliminary illness above referred to we are met with the difficulty that it is unfortunately impossible in the present state of our knowledge to identify malarial pyrexia, and thus to distinguish in all cases this form of pyrexia from non-malarial pyrexia occurring in malarial subjects. The difficulty is connected with our ignorance of the mechanism of production of malarial pyrexia. In the same way we have no means of ascertaining if the pyrexia of blackwater fever is itself of malarial origin or not. It is equally impossible in the present state of our knowledge to determine the significance of the association existing between blackwater fever and malaria.

In all cases of blackwater fever coming under our observation quinine was taken prior to the attack, which it directly preceded (Table 50, p. 247). The relation between the two was usually quite distinct, as for example in Cases 1, 2, 6, and 14a. The interdependence of the two is not, however, a simple one, for in all but five cases (1, 4, 6, 11 and 14) quinine had been administered for one or more days before the appearance of blackwater, but without effect as far as the production of blackwater was concerned. In Case 7a (p. 195) the administration of quinine was observed by the patient to be followed, some days before the attack of blackwater, by a sudden rise of temperature attended with constitutional symptoms closely resembling those accompanying a previous attack of blackwater fever, though no haemoglobinuria made its appearance. It will be noted also that in five cases (4, 8, 10, 12, 14a, Table 50) it was deemed advisable to give quinine shortly after the disappearance of blackwater. In two of these cases (12 and 14a) no relapse occurred. In two other cases (4 and 8) slight relapses occurred, which did not bear any definite relation to the time of administration of quinine. In the remaining case (10) a remarkable series of slight relapses occurred, at first in the absence of quinine, and then still continuing when, after a few days, the administration of quinine was recommenced, without, however, presenting any definite relation to the time of administration of quinine. In this case the administration of quinine did not appear to call forth the relapses, nor did any marked improvement occur during the period when quinine was discontinued.

It has already been pointed out that a possible explanation of the occurrence of haemoglobinuria after quinine in malarial subjects may be the destruction of the red cells, which, as already shown, are capable of taking up quinine from solution (Table 21, p. 41), by the double action of the malarial parasite and quinine, but that it is doubtful if the number of parasites in the red cells of the peripheral blood is large enough to admit of the assumption that the haemoglobin passing into solution in the blood plasma is derived from the haemolysis of these affected red cells, while we are without data pointing conclusively to the occurrence of haemolysis in the spleen or elsewhere. It must, however, be observed that we are not on safe ground in assuming that because quinine is effective in producing blackwater in malarial subjects it would fail to do so in non-malarial patients. Such an assumption is vitiated by the fact that the administration of quinine in large doses is in general use only in the treatment of malaria. In no other disease is the administration of this drug an habitual and generally recognised mode of treatment. Moreover, on the relatively few occasions on which quinine is administered in other diseases it is usually given in much smaller doses ($\frac{1}{2}$ gr. to 2 gr.). Whether haemoglobinuria would occasionally occur if quinine were employed generally in pyrexial affections in doses similar to those employed in malaria, is purely a matter of conjecture.

The above remark is prompted by a point of resemblance between many cases of blackwater fever and the haemoglobinuric attacks produced by haemolytic serum, potassium chlorate and other drugs (p. 128). In the latter cases haemoglobinuria is induced in an animal which has for some time previously been suffering from more or less severe constitutional disturbance. Frequently this is also precisely the condition in which blackwater fever patients exist at or for some days before, the date of appearance of haemoglobinuria. In each case there are present two factors: more or less severe constitutional disturbance, and a toxic agent. Another point of resemblance lies in the circumstance that in both cases blood destruction (changes in the red cells may be obtrusive after the administration of potassium chlorate) has been occurring previous to the appearance of haemoglobinuria resulting in more or less severe anaemia. In both cases an increased blood destruction occurs as soon as haemoglobinuria makes its appearance. It is, however, uncertain whether the blood destruction in these cases is attended with discharge of haemoglobin into the blood plasma, destruction or removal of haemoglobin (cp. Exp. 10, Table 35) occurring so rapidly that the degree of haemoglobinaemia is insufficient to lead to haemoglobinuria, or whether the destruction takes place without any such liberation of haemoglobin into the blood plasma. In the first case the difference between the first process and the haemoglobinaemia of blackwater fever would be only one of degree; in the latter case the difference between the two processes would be a qualitative one. In the former case quinine, potassium chlorate, etc., would act merely by accelerating the rate of discharge of haemoglobin into the blood plasma; in the latter case by inducing a new process. It is of interest to refer in this connection to the circumstance that malaria alone may cause blackwater fever. Twenty-four such cases are described by A. Plehn.*

SUMMARY

1. In the urine in blackwater fever red cells may be found during haemoglobinuria, usually in very small numbers.

2. In blackwater fever the haemoglobinuria which occurs is the result of an accompanying haemoglobinaemia.

3. Sufficient data are not available to determine the situation in which laking of red cells leading to haemoglobinaemia occurs in blackwater fever.

ADDITIONAL NOTE

Redwater in piroplasmosis. Haemoglobinuria in the dog, due to piroplasma canis, is similar in its mechanism of production to that of blackwater in the human subject, that is to say, the haemoglobinuria is attended with and dependent upon haemoglobinaemia. A series of data obtained in experimental piroplasmosis of dogs will shortly be ready for publication.

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* Ätiologie und Pathogenese des Schwarzwasserfiebers, Virch. Arch., 1903, B. 174, S. 514.

VI. THE CONDITION OF THE KIDNEYS IN BLACKWATER FEVER.

The condition of the kidneys during the haemoglobinuria of blackwater fever is of necessity a matter of inference, for a simple uncomplicated attack is not in itself fatal. When death takes place during blackwater fever, this is due to some complication, such as hyperpyrexia (Case 13), suppression of urine (Cases 7a and 11), or severe constitutional disturbance ending in cardiac failure (Case 16), and it may happen that haemoglobinuria has ceased some time before death. In these cases it is necessary to attempt to separate such pathological conditions as are purely representative of the haemoglobinuria from secondary or associated pathological conditions. The post-mortem appearance of the kidneys of animals in which experimental haemoglobinuria, together with severe blood changes, had been produced has already been referred to on p. 128, and our own experiments of a similar but simpler character, unattended with general constitutional disturbance, have been described on pp. 124-127 (figs. 45-51 and 59-60). It will therefore not be necessary to refer further to the appearances presented by the kidneys in these experimental conditions, but reference must be made to the results of post-mortem examination in cases of blackwater fever, omitting, however, any additional description of the kidney changes in suppression of urine following upon blackwater fever (pp. 107-124; figs. 33-40 and 53-56).

In 1882 Kiener and Kelsch* in an article on 'Néphrite paludéenne,' observed in Algeria, described as an early stage of this disease 'congestion hématurique ou hémoglobinurique,' in which the urine contained sometimes dissolved haemoglobin together with brown granular casts and débris, in addition to hyaline casts, leucocytes and red cells, sometimes only red cells, but no dissolved haemoglobin. On section of the kidneys similar casts were found in the uriniferous tubules (loc. cit., p. 285-6; also Plate VI, figs. 8-14). The condition in question would now be called blackwater fever. Further investigations on the histology of the kidneys in blackwater fever were made by Stieda[*] (1893), Marchiafava and Bignami[†] (1901), A. Plehn[‡] (1903), De Haan[§] (1905), and Werner[¶] (1907). In some of the cases recorded, in which death took place on the day on which haemoglobinuria first appeared, it is possible if not probable that the condition of the kidneys would have led to suppression of urine if life had been prolonged. The changes to be considered may be grouped under four heads: the presence of granular material in the lumen of the renal tubules; degenerative and other changes in the renal epithelium; fluid distension of the renal tubules; interstitial changes. The first condition was always present in more or less marked degree. The state of the renal epithelium is not described alike by all observers, some finding the epithelium quite normal, others meeting with cloudy swelling, coagulation necrosis or fatty degeneration, or again the epithelial cells were found loaded with coloured granules. Distension of the renal tubules was usually absent; Werner[‖] describes four cases (Cases 4 and 5 and to less extent Cases 2 and 6) in which this condition was present, but these are not improbably cases in which suppression of urine would have been observed if the patients had survived. Interstitial changes (presence of epithelioid cells and leucocytes) were rarely met with.

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* Les altérations paludéennes des reins, Arch. der Physiologie, 1882, T. 14 p. 278.

In any attempt to apply the above observations to the elucidation of the enquiry, what is the condition of the kidneys in blackwater fever during simple haemoglobinuria, it is necessary to distinguish between the changes in the kidney which are necessarily present and those which when present are to be regarded as secondary or additional pathological changes. We are on safe ground when we infer, from the condition of the urine during blackwater and from post-mortem observations in blackwater fever and in experimental cases, that granular material is present in the renal tubules during haemoglobinuria whether dependent upon blackwater fever or produced experimentally. If haemoglobin is eliminated, not by the glomerulus but by the epithelium of the renal tubule, it is possible that this epithelium may necessarily contain during haemoglobinuria brown granules of haemoglobin as observed by Marchiafava and Bignami (cp. also Marchand† and Afanassiew‡). This is, however, on the whole, improbable; such an appearance seems to have been rarely met with, and was not observed in our experimental cases. In all probability the presence of granular material in the lumen of the renal tubules is the sole change recognisable to the naked eye, which the kidney presents during haemoglobinuria. As regards the degenerative changes occasionally observed in the renal epithelium after death, the circumstance that the urine ordinarily becomes quite normal after the attack of blackwater has ended, no trace of coagulable proteid remaining in the urine after haemoglobin has disappeared, shows that as far as mere haemoglobinuria is concerned the renal epithelium is unaffected, the elimination of haemoglobin being effected by healthy epithelium as occurs in the rabbit during experimental haemoglobinuria (Figs. 44-51 and 59-60). No parenchymatous change of the epithelium necessarily attends the appearance of haemoglobinuria, nor does the constitutional disturbance accompanying blackwater fever seem to be usually accompanied with any secondary affection of the renal epithelium. Whether the granular material in the renal tubules ordinarily causes some obstruction to the flow of urine is uncertain. No diminution, but an increased flow occurred in the experiments on rabbits detailed in Table 35. In blackwater fever patients, however, a diminished flow of urine may be observed not unfrequently during haemoglobinuria (cp. p. 134), The circumstance that interstitial changes are usually absent after death from blackwater fever may be regarded as negativing the necessary association of this condition with the production of haemoglobinuria.

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[*] Einige histologische Befunde bei tropischer Malaria, Centralbl. f. a. Path. u. p. Anat., 1898, B. 4, S. 321.

[†] Malarial haemoglobinuria, Twentieth Century Practice of Medicine, London, 1900, Vol. 19, p. 483.

[‡] Die Nieren beim Schwarzwasserfieber, Arch. f. Schiffs- und Tropenhygiene, 1903, Bd. 7. S. 270.

[§] Die Nieren beim Schwarzwasserfieber, Ibidem, 1905, Bd. 9, S. 22.

[¶] Uber die Nieren beim Schwarzwasserfieber, Ibidem, 1907, Bd. 11, S. 5.

[‖] Loc. cit., pp. 9-11.

We may therefore say that all the evidence available points to the conclusion that the production of haemoglobinuria in blackwater fever is consistent with a normal functional state of the kidney, and is not

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* Loc. cit.

† Über die Intoxication durch chlorsaure Salze, Virch. Arch., 1879, B. 77, S. 455.

‡ Über die pathologisch anatomischen Veränderungen in den Nieren und in der Leber bei einigen mit Hämoglobinurie oder Icterus verbundenen Vergiftungen, Virch. Arch., 1884, Bd. 98, S. 460. Afanassiew regarded these granules as identical with the fragments of red cells seen in his experiments in the circulating blood; these he describes as being taken up by the renal cells and passed on into the lumen of the tubules. Marchand and Ponfick hold similar views.

attended with any pathological condition of the renal epithelium, the only visible change ordinarily present being the appearance of brown granular material in the renal tubules. Whether brown granular material is also of necessity present in the epithelium of the renal tubules during blackwater is uncertain.

It is moreover clear from our observations that an attack of haemoglobinuria does not necessarily damage the kidneys. No albuminuria of renal origin followed the attacks of blackwater fever coming under our observation, in which recovery occurred, and judging from the cases recorded in the literature of this subject, blackwater fever is not a cause of nephritis.

Before concluding this section reference may be made to the circumstance that venereal disease does not seem to predispose to blackwater fever nor to influence its course when present. Particulars on this point were obtained in sixteen cases of blackwater fever, with the result shown in Table 51. On further comparison of the cases in which one or both forms of venereal disease were present with those in which a negative history was obtained, it is found that the severity and duration of the blackwater fever were not obviously greater in the former than in the latter.

TABLE 51. Blackwater fever and venereal disease.

No. of Cases	Syphilis	Gonorrhoea
9	Absent	Present in 3 cases
7	Present	Present in 3 cases

SUMMARY

1. During simple uncomplicated haemoglobinuria of blackwater fever the sole pathological condition existing in the kidneys would appear to be the presence of brown granular material in the lumen of the renal tubules.

2. Venereal disease does not appear to influence either the tendency to blackwater fever or its severity when present.

VII. ICTERUS AND BLACKWATER FEVER.

The statement is frequently made that icterus occurs in blackwater fever. This word, while in its strict sense designating true jaundice due to the presence of bile pigment in the blood plasma and urine, is also sometimes employed loosely to indicate merely a yellowish tinting of the skin and sclerotic coat of the eyeball, not necessarily due to bile colouring matter. The use of the term icterus in describing this latter condition in blackwater fever, without any statement as to whether the urine contains bile pigment or not, is frequent and is apt to be misunderstood. The same indefiniteness of meaning is also apparent in the description of experimental work in which haemoglobinuria has been induced. It would appear, however, that true jaundice, dependent upon bile pigment, is producible together with haemoglobinuria by toluylendiamin,[*] though so far as we know no other drug is capable of producing these two conditions simultaneously. It is asserted by Koch[†] that after the administration of quinine, instead of blackwater fever, an attack of icterus may result, haemoglobin being converted into bile pigment, which appears in the urine.

In the cases of blackwater fever which came under our notice, as also in cases of malaria, the urine was not unfrequently high coloured (brownish amber) after the attack. When the urine obtained during the haemoglobinuria of blackwater fever was acidified and boiled, the liquid obtained on filtration was usually of a light amber colour, though sometimes remaining of a somewhat brownish tint. On testing the filtrate for bile pigment with iodine a negative result was obtained. In the same way a negative result was obtained in the experimental haemoglobinuria of rabbits. In consequence of the absence of bile pigment in the urine it follows that the dark amber or orange colouring matter, obviously derived from the breaking up of haemoglobin, which the blood plasma often presents in blackwater fever and for some time subsequently, and sometimes also in malaria (Table 33, p. 73) is not due to bile pigment.

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[*] G. Joannovicz, Experimentelle Untersuchungen über Ikterus, Zeitschr. f. Heilkunde, 1904, Bd. 25. S. 25. M. Afannassiew, Über die pathologisch-anatomischen Veränderungen in den Nieren und in der Leber bei einigen mit Hämoglobinurie oder Icterus verbundenen Vergiftungen, Virch. Arch., 1884, Bd. 98, S. 460.

[†] Über Schwarzwasserfieber (Hämoglobinurie) Zeitschr. f. Hygiene, 1898, Bd. 30, S. 295. Cp. pp. 321-322 and Case 3, p. 303.

VIII. REMARKS ON THE PROPHYLAXIS AND TREATMENT OF BLACKWATER FEVER.

It is not here proposed to do more than refer briefly to the importance of avoiding malaria in countries in which blackwater fever is prevalent, for the need of adopting the various measures available for preventing the risk of the bites of infected mosquitos and of using quinine as a prophylactic are now well recognised. But a few remarks upon the attitude often taken up towards prophylactic measures will serve to indicate the nature of some of the difficulties, often more or less trivial in aspect, which arise when it is attempted to secure avoidance of malaria. It was, we found, commonly regarded as unnecessary to use mosquito nets until mosquitos had already made their appearance. The presence of mosquitos was, moreover, not unfrequently overlooked. In many cases this appeared to be in part due to the circumstance that those bitten by mosquitos have after the lapse of months or years ceased to suffer from irritative skin lesions as a result of these bites. Mosquito nets, when available, were sometimes not used. Some of the nets in use were in a torn condition and of limited utility. When travelling or shooting mosquito nets were not unfrequently left behind, though the night would be spent in the neighbourhood of a river where mosquitos abounded, and this in spite of warnings afforded by the consequences of such neglect. The importance of taking a course of quinine as a prophylactic after being bitten by mosquitos is not recognised, and this drug is not taken until an attack of malaria has supervened, or until several malarial attacks of increasing severity have occurred. The necessity of avoiding the formation of collections of water in the neighbourhood of houses, or of filling up or draining places in which, during the rainy season, mosquitos may breed is generally unknown to the white population, but the Government medical officers have taken steps to secure better sanitary conditions in such cases. Many of the situations in which habitations were placed involved unnecessary risk of exposure to malaria.

The administration of quinine to a patient suffering from malaria who has previously suffered from blackwater fever presents a difficulty for which no general rule can be laid down. In some cases where the malarial condition is not showing signs of improvement, or is becoming steadily worse, the possible risk of inducing blackwater by giving quinine may be less than that of withholding it. Sometimes the decision may be left to the patient, but in most cases the responsibility of deciding upon the treatment will necessarily rest with the medical officer. We are of opinion that the general avoidance of quinine in malarial cases with a view to escaping the possible risk of blackwater fever would expose patients to a much greater risk of fatal issue from complications of malaria. The degree of risk involved in giving quinine shortly after an attack of blackwater fever may be judged from a glance at Table 50 (Cases 4, 8, 12, 14a). It is scarcely necessary to add that when quinine is given its use should be continued as long as splenic enlargement or other sign of malaria is observable.

During the attack of blackwater fever quinine is probably best avoided, but when haemoglobinuria has ceased and the temperature has become normal, the treatment of the accompanying malarial condition may be commenced with ½ gr. daily doses of quinine; very small doses appearing to be much less likely to cause haemoglobinuria than larger doses. This amount may be gradually increased to 5 gr. a day (cp. Case 14a, Table 48).

Of the treatment of the general symptoms of blackwater fever nothing will be said, since these are dealt with by the same methods which are employed when they occur in other diseases. Our experience does not enable us to express any opinion as to the value of the numerous empirical methods for the treatment of blackwater fever which have been put forward by various authors. A few remarks may, however, be made on the treatment of a complication which is peculiar to blackwater fever and is a frequent cause of death, namely, suppression of urine. It has already been pointed out that the tendency to plugging of the renal tubules with granular material, which is purely mechanical in its action, appears less likely to occur when the flow of urine is rapid than when it is sluggish. For this reason in all cases of blackwater fever, and especially when the amount of urine secreted is small, which is likely to be the case when the patient has been sweating profusely, as often happens, or exhibits signs of cardiac depression, the secretion of urine by the patient should be encouraged by the administration of a copious amount of fluid to drink and by the use of tea, caffeine, digitalis or other diuretics. If suppression has supervened these measures may be continued for a time, but if suppression continues the question arises whether any attempt to relieve the blocking of the renal tubes by providing an outlet mechanically by incision of one of the kidneys should be made. Should such a measure have been attempted in Case 7a, for example, in which the general condition was good almost to the end? Up to the present this procedure, first suggested by Werner,* appears to have been adopted in suppression of urine in blackwater fever only on two occasions. In the first case,† on the third day of suppression nephrotomy was performed, the flow of urine containing abundance of albumin being re-established, but subsequently anuria reappeared and death occurred. In the second case‡ nephrotomy was performed after five days' suppression of urine; the operation resulted in the re-establishment of a profuse secretion of urine, but death occurred subsequently from progressively increasing weakness. It is clear, on reference to Fig. 62 (p. 130), that if the blocking of the renal passages is sufficiently extensive no mere incision of the kidney would be of much use, but if the blocking is chiefly confined to the ducts of Bertini, as probably occurs in most cases and was observed in Cases 7a and 11 (Figs. 33-40 and 53-56), then nephrotomy, if the patient's strength is maintained, offers a means of re-establishing the flow of urine. It does not, however, follow that this will necessarily lead to recovery, for though cases are on record in which after several days' suppression in blackwater fever re-establishment of the flow of urine has occurred spontaneously, yet, nevertheless, death occurred from other complications; two such cases are recorded by F. Plehn. The effect of incision is of course limited, as regards the outlet which it provides for the escape of urine, to only a small fraction of the kidney incised, but it may be pointed out that a fraction of one kidney not exceeding one half to one quarter of the total kidney mass is sufficient to carry on the work ordinarily performed by the two kidneys.§ The best time for carrying out nephrotomy is probably before the suppression, determined by catheterisation, has lasted more than twenty-four to forty-eight¶ hours, it being, of course, assumed that the general condition of the patient is sufficiently good to permit of operation.

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* Ist bei Schwarzwasserfieberanurie die Nephrotomie indiziert? Deut. med. Wochenschr., 1901, No. 42.

† Ziemann, Monographie über Malaria, Mense's Handbuch der Tropenkrankheiten, Bd. III, S. 585-6.

‡ Reported by Kruger. See Werner, Über die Nieren beim Schwarzwasserfieber, Arch. f. Schiffs- u. Tropenhyg., 1907, Bd 11, S. 5.

§ R. M. Pearce, The influence of reduction of kidney substance upon nitrogenous metabolism, Journ. of Exp. Medicine, 1905, Vol. 10, p. 632.

¶ Cp. Werner, loc cit., p. 17.

IX. CLINICAL RECORDS.

Before giving the clinical records of the patients coming under observation, a few words may be said, by way of introduction, concerning the general features presented by the attacks of blackwater fever from which they suffered. For convenience of reference, a synopsis is given in Table 52, p. 248. The observations have already been for the most part collected in tabular form, as indicated below. Some of the particulars supplied to us by the patients, for example, the statements made as to past attacks of malaria, it was not in our power to verify.

The records are of necessity more or less incomplete. In some cases delay occurred in reaching the patient, either because information could not be sent to us sufficiently early, or, more frequently, because of the slowness of travelling, especially during the rainy season. The simultaneous occurrence of two or more cases at considerable distance apart caused difficulty also on more than one occasion.

The number of attacks of blackwater fever investigated was twenty. These occurred in seventeen different individuals, all males, eleven being white men, four Indians, one Eurasian and one Chinese. Only one attack occurred in a female during our stay in Nyasaland, this was, however, not brought under our observation.

For convenience of reference and comparison dates are omitted, and the course of the attacks is described under the headings 1st day, 2nd day, etc. (cp. Table 50, p. 247). One attack occurred in January, six in February, four in April, one in May, two in June, three in September, two in October and one in December. The rainy season extends from November to March.

All the patients, except Case 5, stated that they had suffered from malaria some time before the attack (cp. Table 49, p. 161). In all except four (Cases 1, 4, 13 and 14) of the attacks the patients were regarded as suffering from malaria before the appearance of blackwater. Malarial parasites were found in the peripheral blood on only one occasion (Case 3), as is indicated in Table 46 (p. 151).

In all the twenty attacks investigated quinine had been taken before the onset of haemoglobinuria, of which it appeared to be a determining factor, especially in attacks 1, 2, 3, 6, 7, 11, 12, 13, 14 and 14a, This relationship is exhibited in tabular form in Table 50 (p. 247), and is further considered in Section V, pp. 136-165.

The duration of the attack of haemoglobinuria was usually short, lasting from three or four hours to four days. (Table 50). Relapses occurred in three cases (4, 8 and 10). In the first two a single relapse occurred respectively ten days and two days after the original attack ; in the last case a remarkable series of small relapses, about fifteen in number, occurred during the ten days following the original attack, and bore no obvious relation to the quinine taken during this period (Table 50). Three of the patients (6, 7 and 14) suffered from another attack of blackwater fever after the lapse of a period varying from two to three and a half months.

The severity of the constitutional symptoms varied considerably in different attacks, being insignificant, for example, in Case 7, while severe collapse lasting about twenty hours occurred in Case 2. Vomiting was severe in attacks 3, 6a, 9, 14a, 16 and 17. Pain in the back was common, but was not severe. Rigors occurred at the onset of the attack in Cases 1, 2, 3, 5, 8, 9, 10, 12, 14 and 14a. Cramps in the calf muscles were severe in Case 2. Illness of several days' duration preceded the attack of blackwater, except in 1, 4, 13 and 14a.

The complications observed were hyperpyrexia (Case 13), suppression of urine (Cases 7a and 11) and vomiting attended with extreme weakness (Case 16). All these complications terminated in death.

The blood obtained by pricking the finger tip was generally remarkably watery in aspect. Haemoglobinaemia was usually present during haemoglobinuria in the cases in which this point was investigated (3, 7a, 10, 14, 15 and 17, cp. Table 34, p. 75), the highest amount observed being 0·95 per cent. Methaemoglobin was also observed in relatively small amount during blackwater in most cases in the blood plasma, which was dark orange in colour in every attack, except 5, 6a and 9, in which it was light yellow (Cases 1 and 13 were not examined)

The reaction of the red blood cells to quinine in vitro was noted in Cases 3, 4, 5, 7, 7a, 8, 10, 11, 14, 14a and 15 ; it was found not to exhibit any marked alteration (Table 24, p. 46).

The percentage of haemoglobin in the urine lay, in the cases in which this could be determined, between 0·2 per cent. and 3·8 per cent. (Table 43, p. 136). The total amount of haemoglobin, lost in the urine, ranged between 1·5 g. and 75 g. (Table 45, p. 141). The total amount of dissolved haemoglobin entering the blood plasma could not be determined (cp. p. 143). Methaemoglobin was also usually observed in the urine in relatively small amount. Red blood cells were found in the urine (Table 43). Casts, generally granular in character, were always present (Section IV, p. 94; Figs. 21 to 32). The urine before and after the attack was frequently high coloured, as in Cases 2 and 7, for example. Bile pigment was not found in the urine.

In Cases 7a and 11 (suppression of urine) and in Case 16 (death seven days after blackwater had ceased) some of the renal tubules, in particular the collecting tubules, were found to be plugged with coarsely granular casts (Figs. 33-40 and 53-56) resembling those present in the urine in Case 7a (Figs. 29 and 32). The renal epithelium did not show any marked degenerative changes. The condition of the urine during nine days' suppression is given on p. 199 (Case 7a) and on pp. 220-221 (Case 11).

The spleen was observed to be enlarged, usually markedly so, in all cases except 13 and 15, in which no information as to its condition was available (Table 47, p. 154)

BLACKWATER FEVER. CASE 1.

Male, twenty-nine years of age. Engineer. European.

Has lived in Nyasaland for the last five years. During this period he has suffered a good deal from fever. Has had one previous attack of blackwater fever about three years ago. He does not take quinine regularly. About a month ago he suffered from low intermittent fever. He stated that he had an attack of fever each alternate afternoon about 3 p.m. These relapses went on for about a fortnight, and during this time he took no quinine. After these attacks of fever had stopped, he took five grains of quinine daily for three or four days. During the next fourteen days he felt very well, and took no quinine

On the 1st day of the present illness he got up feeling very well, and took five grains of quinine. About noon he suddenly became dizzy and had a rigor, and shortly afterwards he passed blackwater.

2nd day. In the morning patient was comfortable, vomiting was slight, and the urine, which had begun to clear about noon, was still deeply tinged red. Pulse 84. Temperature normal. The spleen extended one and a half inches below the costal margin. The liver was also enlarged. At 9 p.m. patient was not quite so well. Temperature 103° F. The urine had become much darker again.

3rd day. Patient much better; the vomiting had ceased and the urine was not so deeply coloured. Temperature 99·5° F.

4th day. Patient better; the urine was amber coloured and the temperature normal. Subsequently the patient's recovery was uninterrupted.

Condition of blood.—On the 2nd day an examination of the patient's blood was made at 4-30 p.m. No malarial parasites and no pigmented leucocytes were found.

3rd day. Blood was examined at 10 a.m. No malarial parasites and no pigmented leucocytes found.

4th day. Blood films were examined again to-day, and no malarial parasites or pigmented leucocytes were found.

7th day. No malarial parasites or pigmented leucocytes were found in films made from the patient's blood to-day.

15th day. No isolysin could be discovered in patient's blood plasma. No malarial parasites or pigmented leucocytes were found in the blood films.

Condition of urine.—On the 1st day, at 2 p.m., the urine was of a dark red colour.

2nd day. The urine was not quite so deeply coloured at noon to-day, but at 9 p.m. it became of a dark red colour.

3rd day. The urine was of a reddish colour to-day, but became lighter in the evening. On spectroscopic examination the bands of oxyhaemoglobin were seen. Methaemoglobin bands were absent. A few red cells were present in the urine.

4th day. Urine was amber coloured.

15th day. The urine was acid, sp. gr. 1·010, amber coloured; no deposit on centrifugalising, and no precipitate on acidifying and boiling.

Blackwater Fever. Case 2.

Male about forty years of age. Planter. European.

Has been in Nyasaland for thirteen years. His first attack of blackwater fever occurred eight years ago; was ill with 'fever' for a fortnight, then took forty grains of quinine, after which blackwater appeared. Since then has had several attacks of malaria, but has not suffered again from blackwater fever. Has passed small amounts of pus in his urine for last twelve months, and occasionally has passed bright blood at the end of micturition.

Eight days ago was drenched, and could not change his clothing for some hours. Yesterday took five grains of quinine, had to pass a catheter, being unable to void urine naturally.

To-day (first day) temperature became raised; patient suffers from vomiting and diarrhoea, and is very restless. Has painful cramps in muscles of legs. Took five grains of quinine.

2nd day. Still restless but somewhat improved in morning. At 10 a.m. had six grains of quinine bihydrochloride hypodermically, his temperature being then 98·4° F. At 11-15 a.m. again restless, temperature 103·5° F., vomited. At 12-0 mid-day passed 150 c.cm. of very dark chocolate-coloured urine. Pulse now very feeble and rapid; shivering; extremities cold. At 12-30 p.m., temperature 105° F.; at 1-30 p.m., temperature 101° F. Then commenced to perspire freely, and condition improved. Spleen projects about 3½in. below costal margin. Liver cannot be felt. Heart and lungs normal.

3rd day. Condition much improved. Skin has become sallow. Urine contained chocolate-coloured deposit until mid-day, when this disappeared and dark amber urine was passed.

Subsequently patient's condition steadily improved. The urine continued free from haemoglobin, no relapse occurring. Pus in urine continued as before.

Condition of blood.—On the 1st day, before blackwater appeared, an examination of patient's blood was made at 4 p.m. The blood plasma was of a dark orange colour with a slight greenish tint, and gave, in a column 18 mm. high, excessively faint oxyhaemoglobin bands, the amount of dissolved haemoglobin being about 0·13 per cent. No isolysin was present. No malarial parasites could be found in blood smear.

2nd day. Blood examined at 9 a.m. and again at 2-15 p.m. On each occasion the plasma was found to be of a dark orange colour, with a slight greenish tint, containing about 0·13 per cent. of dissolved haemoglobin. No isolysin present. No malarial parasites in blood smear.

3rd day. Blood examined at 2·30 p.m. Plasma of dark orange colour, with slight greenish tint. As before, only excessively faint oxyhaemoglobin bands could be recognised in a column 18 mm. high, the amount of dissolved haemoglobin being about 0·13 per cent. No isolysin present. No malarial parasites in blood smear.

Condition of urine.—Prior to the appearance of blackwater the urine was stated to be of a yellow colour; none was obtainable before the onset of blackwater. Characters of urine are given in the following table:—

Date.	Amount.	Specific Gravity.	Reaction.	Colour.	Amount and appearance of deposit.	Spectroscopic examination.
day, 12.0 mid day	80 c.c.	1·022	Neutral	Chocolate coloured	5 c.c. chocolate brown	No dissolved haemoglobin recognisable
" 6.45 p.m.	50 "	1·019	"	"	7 " "	" "
" 11.30 "	118 "	1·022	"	Dark amber coloured	3·5 c.c "	" "
day, 3.20 a.m.	125 "	1·012	"	"	1 c.c. whitish	" "
" 6.30 "	85 "	1·014	"	"	2 " "	" "
" 9.30 "	136 "	1·014	"	"	3 " "	" "
" 12.0 p.m.	115 "	1·018	"	"	6 c.c. "	" "
" 3.30 "	115 "					
" 4.15 "	60 "					
day, 2.0 a.m.	170 "					
" 3.45 "	85 "					
" 6.30 "	195 "					
" 9.0 "	210 "					
" 11.45 "	220 "					

The first two specimens of urine presented an abundant deposit, consisting chiefly of red blood cells and stromata, and a dark brown granular precipitate, the latter being one third to one quarter of the volume of the former. In addition a few epithelial cells were seen, and also bacilli, mostly 3μ to 4μ long, somewhat less numerous than the red cells. On centrifugalising the urine, the brownish precipitate was separated with difficulty, and a turbid upper liquid, light brown in colour, showing on spectroscopic examination no haemoglobin bands in a layer 18 mm. high, and giving on acidifying and boiling about one-twentieth column of a whitish brown precipitate, was obtained. From the supernatant liquid no haemin crystals could be obtained, but on adding tincture of guaiacum and hydrogen peroxide a blue colour was obtained. The deposit in the second specimen of urine contained in addition a large number of pus cells (about one to every eight red cells), some of which had ingested red cells, and a few blood casts and brown granular casts.

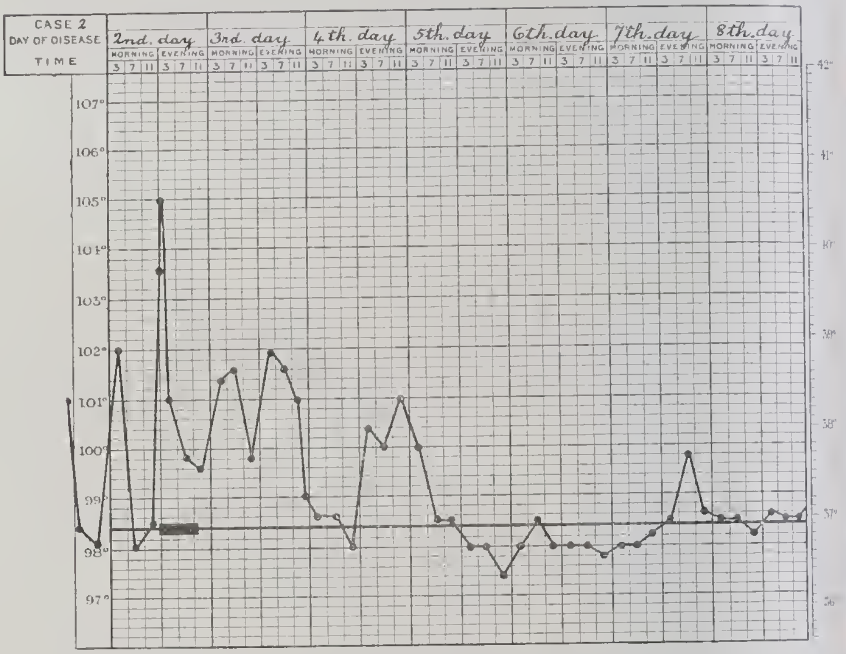

FIG. 71. Blackwater Fever, Case 2. Temperature Chart.

In the succeeding four specimens of urine the deposit consisted chiefly of pus cells, some of which contained red cells or brown pigment; also in smaller amount red cells, partly or wholly decolourised, and granular brown pigment, the latter preponderating over the former; brown granular pigment casts, occasionally hyaline casts; squamous epithelial cells; cylindrical epithelial cells; and bacteria, present in abundance in the urine immediately after being passed. The supernatant liquid was free from odour of decomposition, was turbid, of a dark amber colour, neutral in reaction, and gave on acidifying and boiling one-fifteenth column of coagulated proteid of a light brown colour; on adding tincture of guaiacum and hydrogen peroxide a faint blue colour was obtained; no bands of haemoglobin or urobilin were seen on spectroscopic examination.

In the remaining specimens of urine the deposit consisted almost entirely of pus cells, sometimes in shreddy masses. In addition granular casts, epithelial cells and abundant bacteria were present.

A week after recovery the urine still contained pus cells and bacilli.

Owing to the absence of oxyhaemoglobin bands in the centrifugalised urine at the time of examination, and the presence of pus, it was impossible to determine the amount of dissolved haemoglobin which may have been originally present in the urine.

BLACKWATER FEVER. CASE 3.

Male, forty seven years of age. Engineer. European.

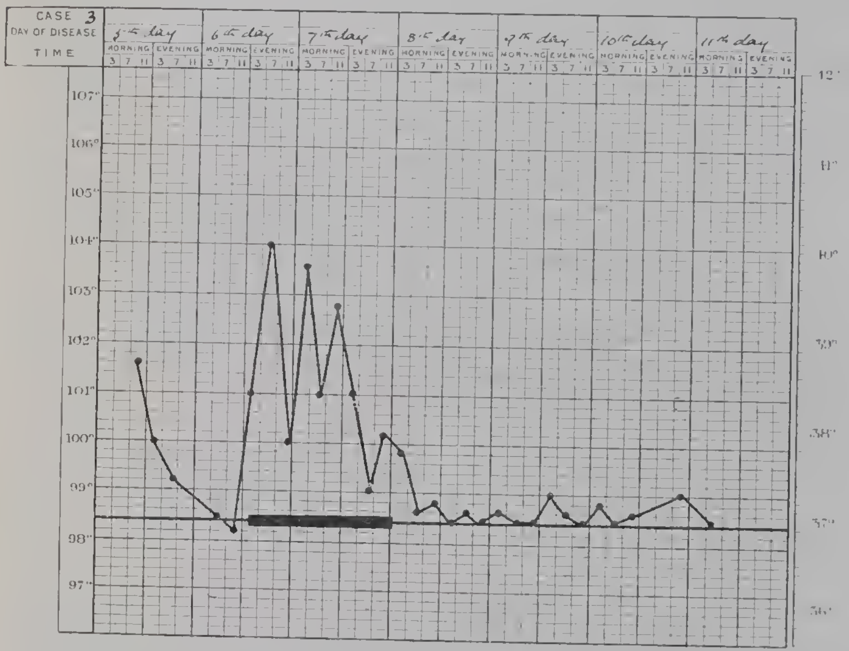

FIG. 72. Blackwater Fever, Case 3. Temperature Chart.

First came to Nyasaland ten years ago. Had fever every two or three months during the first four years of his residence in Nyasaland.

Eight years ago he had his first attack of blackwater fever. This attack was very severe, and he continued to pass blackwater for five days. The next two years he suffered from a good deal of fever, and had three more attacks of blackwater fever. He stated that after each of these attacks he had a relapse. During the next six years he had but little fever, and no further attack of blackwater fever. He used to take about twenty grains of quinine a week during the first five years he spent in Nyasaland, but has taken hardly any for the last five years.

During the last two months he has been living in a very wet camp, but remained comparatively well until about a week ago, when he had a severe attack of fever. This passed off, but was followed by another two days later, and by still another on the next day. He took five grains of quinine on the third day of his illness and ten grains on the fourth day, previously not having taken any for over a year. On the fifth day he was admitted to hospital, and given five grains of quinine. T. 101·6° F.

6th day. Slept badly, perspired freely during the night. Quinine bihydrochloride in solution was administered in five grain doses at 7 a.m., 9 a.m. and noon. At 2 p.m. he slept for an hour, but awakened feeling chilly. At 3 p.m. T. 101° F., and at 3-30 p.m. he passed red urine. At 6 p.m. T. 104° F., complained of pain in chest and legs. Had a good deal of retching, bringing up a little bile-stained fluid. At 7 p.m. T 100° F., pulse 104. Complained of flatulency, skin was of a very distinct lemon yellow tint. Spleen easily palpable.

7th day. Troubled greatly with flatulency. Vomiting was very troublesome. At 6 a.m. T. 99° F., pulse 78. At 9 p.m. T. 100·2° F., the vomiting was less troublesome.

8th day. Patient much better. T. 100° F. at 6 a.m.

9th day. Felt quite well. Skin still of a lemon yellow colour, spleen enlarged and hard, and extended three fingers' breadth below costal margin.

Condition of blood.—5th day, at 3-30 p.m. Patient's oxalated plasma was of a dark orange colour with no red tint. Oxyhaemoglobin bands were faint, the blood plasma containing 0·16 per cent. of oxyhaemoglobin. No autolysin present. A few malarial parasites, but no pigmented leucocytes, were found in the blood.

6th day. At 6 p.m. patient's oxalated plasma was of a dark orange colour with a distinct reddish tint. Oxyhaemoglobin bands were distinct, the blood plasma containing 0·57 per cent. of oxyhaemoglobin. No autolysin present. No malarial parasites or pigmented leucocytes were found in the blood. At 10 p.m. patient's oxalated plasma was of a dark orange colour with a distinct reddish tint. Oxyhaemoglobin bands were distinct, the blood plasma containing 0·4 per cent. of oxyhaemoglobin. No autolysin present in the plasma. No malarial parasites or pigmented leucocytes were found in the blood.

7th day. At 11-45 a.m. patient's oxalated plasma was light yellow coloured, with no reddish tint. Oxyhaemoglobin bands were faint, the blood plasma containing 0·8 per cent. of oxyhaemoglobin. No autolysin was present in the plasma, and no malarial parasites or pigmented leucocytes were found in the blood.

The following observations were made at 37° C. with the patient's red blood cells suspended in 0·9 per cent. sodium chloride solution to which quinine bihydrochloride had been added:

No. of experiment.	COMPOSITION OF MIXTURE OF RED BLOOD CELLS AND QUININE SOLUTION.			Haemolysis.
	2·5 of % emulsion of red blood cells	0·9 % solution of sodium chloride	1·92 % solution of quinine bihydrochloride	
1	0·4 c.c.	2·0 c.c.	0·1 c.c.	Complete in ½ hour
2	0·5 c.c.	2·4 c.c.	0·1 c.c.	Complete in 1 hour
3	0·65 c.c.	3·0 c.c.	0·1 c.c.	Complete in 1½ hours
4	0·8 c.c.	4·6 c.c.	0·1 c.c.	Complete in 2½ hours

8th day. At 10 a.m. patient's oxalated plasma was orange coloured. Oxyhaemoglobin bands were faint, the blood plasma containing 0·08 per cent. of oxyhaemoglobin. On examination with the aid of the haemocrit, the patient's blood was found to contain 20 per cent. by volume of red cells. A haemoglobinometer reading of 30 divisions of Von Fleischl's scale was obtained (equivalent to 0·09 per cent. of wet red cells). A haemocytometer enumeration gave the following figures:—Red corpuscles, 2,612,500 per mm.^{3}; leucocytes, 5,470 per mm.^{3} Red cell index = 0·58. No autolysin present. No parasites and no pigmented leucocytes were seen in the blood.

Date and time.	Amount.	Sp. gr.	Reaction.	Colour.	Appearance and amount of deposit.	Spectroscopic examination.	Amount of haemoglobin passed.
5th day	—	—	—	Amber coloured			
6th day, 3.30 p.m.	62 c.c.	1·013	Alkaline	Dark red	Slight whitish deposit consisting of colourless granular matter and granular casts. No red cells.	Oxyhaemoglobin bands present, but no methaemoglobin bands visible.	1·4 % (corresponding to 0·9 gr. of haemoglobin).
,, 5.15 ,,	86 c.c.	1·010	Neutral	,,	,, ,,	,,	1·2 % (corresponding to 1 gr. of haemoglobin).
,, 6.0 ,,	86 c.c.	1·011	Alkaline	,,	,, ,,	,,	1·2 % (corresponding to 1 gr of haemoglobin).
8.0 ,,	110 c.c.	1·012	,,	,,	As above, but the deposit is slightly larger in amount.	,,	1·3 % (corresponding to 1·2 gr. of haemoglobin). Total haemoglobin passed during the day equals 4·2 gr.
7th day, 2.0 a.m.	105 c.c.	1·023	,,	Dark reddish brown	Small amount of deposit consisting of granular debris and casts with crystals of triple phosphates.	,,	1·3 % (corresponding to 1·4 gr. of haemoglobin).
,, 4.30 ,,	165 c.c.	1·019	,,	Porter coloured	,, ,,	,,	1·3 % (corresponding to 2·3 gr. of haemoglobin .
,, 7.45 ,,	125 c.c.	1·017	Neutral	,,	Small amount of deposit consisting of granular casts with apparently every transition to very granular epithelial casts. Also a few very granular single renal cells mostly columnar, a few cubical. A small amount of free granules about 2 μ in diameter. No bacteria; about 10 red cells seen, mostly completely decolourised. Occasionally a pigmented mass.	,,	0·73 % (corresponding to 1·2 gr. of haemoglobin
,, 10.30 ,,	82 c.c.	1·018	Alkaline	,,	,, ,,	,,	0·73 % (corresponding to 0·8 gr. of haemoglobin).
,, 2.15 p.m.	210 c.c.	1·013	,,	Brown	Slight deposit on centrifugalising, showing same characters as above.	,,	0·1 % (corresponding to 0·2 gr. of haemoglobin).
,, 6.30 ,,	203 c.c.	1·014	Neutral	Light red	Slight whitish deposit.	,,	0·1 % (corresponding to 0·2 gr. of haemoglobin).
,, 9.30 ,,	135 c.c.	1·015	,,	Porter coloured	About $\frac{1}{20}$th column of whitish deposit.	,,	1 % (corresponding to 1·4 gr. of haemoglobin). Total haemoglobin passed during the day equalled 7·5 gr.
8th day, 2.0 a.m.	142 c.c.	1·015	Acid	Brown	Loose whitish deposit, about $\frac{1}{4}$ column, consisting of granular casts and masses, also epithelial casts and cells as previously. Some reddish brown coloration of the casts and masses.	,,	0·1 % (corresponding to 0·2 gr of haemoglobin).
,, 7.0 ,,	148 c.c.	1·014	,,	Light brown	Loose whitish deposit of about $\frac{1}{3}$ column.	No bands.	
,, 10.30 ,,	190 c.c.	1·012	,,	Brown	Whitish deposit about $\frac{1}{4}$ column.	,,	
,, till midnight	816 c.c.	1·016	,,	Amber coloured	Very slight white deposit.	,,	
9th day	1480 c.c.	1·016	,,	,,	,, ,,	,,	

Date and time.		Amount.	Sp. gr.	Reaction.	Colour.	Appearance and amount of deposit.	Spectroscopic examination.	Amount of haemoglobin passed.
5th day		—	—	—	Amber coloured			
6th day,	3.30 p.m.	62 c.c.	1·013	Alkaline	Dark red	Slight whitish deposit consisting of colourless granular matter and granular casts. No red cells.	Oxyhaemoglobin bands present, but no methaemoglobin bands visible.	1·4 % (corresponding to 0·9 gr. of haemoglobin).
,,	5.15 ,,	86 c.c.	1·010	Neutral	,,	,, ,,	,,	1·2 % (corresponding to 1 gr. of haemoglobin).
,,	6.0 ,,	86 c.c.	1·011	Alkaline	,,	,, ,,	,,	1·2 % (corresponding to 1 gr. of haemoglobin).
	8.0 ,,	110 c.c.	1·012	,,	,,	As above, but the deposit is slightly larger in amount.	,,	1·3 % (corresponding to 1·2 gr. of haemoglobin).
								Total haemoglobin passed during the day equals 4·2 gr.
7th day,	2.0 a.m.	105 c.c.	1·023	,,	Dark reddish brown	Small amount of deposit consisting of granular debris and casts with crystals of triple phosphates.	,,	1·3 % (corresponding to 1·4 gr. of haemoglobin).
,,	4.30 ,,	165 c.c.	1·019	,,	Porter coloured	,, ,,	,,	1·3 % (corresponding to 2·3 gr. of haemoglobin).
,,	7.45 ,,	125 c.c.	1·017	Neutral	,,	Small amount of deposit consisting of granular casts with apparently every transition to very granular epithelial casts. Also a few very granular single renal cells mostly columnar, a few cubical. A small amount of free granules about 2 μ in diameter. No bacteria; about 10 red cells seen, mostly completely decolourised. Occasionally a pigmented mass.	,,	0·73 % (corresponding to 1·2 gr. of haemoglobin).
,,	10.30 ,,	82 c.c.	1·018	Alkaline	,,	,, ,,	,,	0·73 % (corresponding to 0·8 gr. of haemoglobin).
,,	2.15 p.m.	210 c.c.	1·013	,,	Brown	Slight deposit on centrifugalising, showing same characters as above.	,,	0·1 % (corresponding to 0·2 gr. of haemoglobin).
,,	6.30 ,,	203 c.c.	1·014	Neutral	Light red	Slight whitish deposit.	,,	0·1 % (corresponding to 0·2 gr. of haemoglobin).
,,	9.30 ,,	135 c.c.	1·015	,,	Porter coloured	About $\frac{1}{20}$th column of whitish deposit.	,,	1 % (corresponding to 1·4 gr. of haemoglobin).
								Total haemoglobin passed during the day equalled 7·5 gr.
8th day,	2.0 a.m.	140 c.c.	1·015	Acid	Brown	Loose whitish deposit, about ¼ column, consisting of granular casts and masses, also epithelial casts and cells as previously. Some reddish brown coloration of the casts and masses.	,,	0·1 % (corresponding to 0·2 gr. of haemoglobin).
,,	7.0 ,,	148 c.c.	1·014	,,	Light brown	Loose whitish deposit of about ⅓ column.	No bands.	
,,	10.30 ,,	190 c.c.	1·012	,,	Brown	Whitish deposit about ¼ column.	,,	
,,	till midnight	816 c.c.	1·016	,,	Amber coloured	Very slight white deposit.	,,	
9th day		1480 c.c.	1·016	,,	,,	,, ,,	,,	

The subsequent specimens of the urine were amber coloured, contained no deposit, and gave no precipitate on acidifying with acetic acid and boiling.

BLACKWATER FEVER. CASE 4.

Male, about thirty-five years of age. Clerk. Indian.

Has lived in Nyasaland for the last seven years, and has had a good deal of malaria during this time. About ten months ago he had an attack of blackwater fever, since which, except for occasional severe headaches, he has been perfectly well. He has hardly taken any quinine since this attack of blackwater fever.

On the first day of the present illness he had a very severe headache, and felt feverish about mid-day to-day. At 3 p.m. T. 103° F. He took ten grains of quinine. At 7 p.m. he took five grains of quinine. At 9 p.m. he had a slight rigor. During the night he states that he had great frequency of micturition, passing water about twenty-five times in very small quantities.

2nd day. When he got up this morning he found that his urine was of a dark red colour. He passed urine five times to-day, the colour increasing until mid-day, and then gradually decreasing. Vomited once this morning.

3rd day. Headache still troublesome; no vomiting; the urine cleared up this morning.

4th day. Patient better; the urine was amber coloured.

5th day. Patient practically well. T. 98·2° F. The urine was amber coloured. Spleen large, reaching one and a half inches below the costal margin.

6th day. Five grains of quinine bihydrochloride was given by the mouth, and three grains subcutaneously. The urine remained amber coloured.

From the 7th to the 13th day the patient remained well, and the urine continued to be amber coloured. Three grains of quinine bihydrochloride were administered thrice daily during this period.

14th day. The patient commenced to feel unwell again last evening. This morning he was worse, and had lumbar pains, which did not descend into the testicles or thighs. He vomited several times. He took one dose of three grains of quinine this morning.

At mid-day he had a slight rigor, and about 2 p.m. he passed Burgundy-red urine. At 3 p.m. T. 104·2° F. Spleen enlarged and easily palpable, extending about two fingers' breadth below the costal margin.

15th day. Patient was better to-day, and the urine was amber coloured.

16th day. Patient quite well to-day. His recovery continued uninterruptedly.

Condition of blood.—5th day. At 10 a.m. patient's oxalated plasma was of a dark orange colour with no red tint, containing 0·06 per cent. of oxyhaemoglobin. No methaemoglobin bands were observed. No isolysin and no iso-agglutination of the red cells was present. A haemocytometer enumeration gave the following figures:—Red cells = 4,140,000 per mm.^{3} No malarial parasites and no pigmented leucocytes were found in the blood.

6th day. On examination of blood films, no malarial parasites or pigmented leucocytes were seen.

16th day. At 10 a.m. patient's oxalated plasma was of a light yellow colour with no red tint, containing 0·13 per cent. of haemoglobin. No autolysin present.

The following observations were made at 37° C. with the patient's red blood cells suspended in 0·9 per cent. sodium chloride solution to which quinine bihydrochloride had been added:—

No. of experiment.	COMPOSITION OF MIXTURE OF RED BLOOD CELLS AND QUININE SOLUTION.			Haemolysis.
	2·5 % emulsion of red blood cells	0·9 % solution of sodium chloride	1·92 % solution of quinine bihydrochloride	
1	0·65 c.c.	3·0 c.c.	0·1 c.c.	Partial at end of 3 hours.
2	0·8 c.c.	3·6 c.c.	0·1 c.c.	Almost complete at end of 3 hours.
3	1·05 c.c.	4·6 c.c.	0·1 c.c.	Trace at end of 3 hours.

Condition of urine.—On the 1st day, in the evening the urine was of a dark red colour.

2nd day. The urine was porter coloured all day.

3rd day. The urine was amber coloured.

4th day. The urine was dark amber colour, sp. gr. 1·018, no deposit on centrifugalising, no oxyhaemoglobin bands, and no precipitate on acidifying with acetic acid and boiling.

From the 5th day until the 13th day the urine possessed the same characters as on the 4th day.

14th day. The urine became red again this afternoon about 2 p.m.

15th day. The red colour of the urine continued all day, but began to clear towards evening.

16th day. The urine was amber coloured and slightly alkaline. On centrifugalising there was a slight deposit, consisting almost entirely of granular casts and masses. No red cells nor stromata. A few columnar epithelial cells and triple phosphate crystals. Slight precipitate on acidifying with acetic acid and boiling.

17th day. The urine was amber coloured, and gave no precipitate on acidifying and boiling.

Subsequent specimens of the urine were amber coloured and normal.

BLACKWATER FEVER. CASE 5.

Male, thirty-seven years of age. Cook. Goanese.

Has been in Nyasaland for ten months. States that he has not suffered from attacks of malaria. Six months ago had an attack of blackwater fever; was ill for one month, the haemoglobinuria extending over ten days and gradually subsiding, without any relapses occurring. Has not had any other illnesses.

1st day. Illness commenced with rigor and profuse sweating. Anorexia, but no vomiting. Has not had quinine.

2nd day. Illness continues. Took ten grains of quinine.

3rd day. Rigor commencing at 6 a.m., accompanied by severe constitutional disturbance. Passed port-wine-coloured urine. Has lumbar pain (bilateral) not extending into testes or groins. Took ten grains of quinine.

4th day. Urine became lighter in colour, was finally faintly red coloured. Heart and lungs normal. Spleen enlarged, projecting at end of respiration about one inch below costal margin. Splenic dulness increased. Liver dulness normal; liver not palpable. Is extremely weak. Took ten grains of quinine.

5th day. Urine at first red, became later dark amber coloured with no red tint. Is very anaemic and weak. Pain at epigastrum. Complexion sallow. Quinine discontinued.

Subsequently patient's recovery was uninterrupted. No relapse occurred.

Condition of blood.—5th day. Blood smear towards close of haemoglobinuria, at 1 p.m., contained no malarial parasites.

6th day. Examination made at 10 a.m., twelve hours after haemoglobinuria had ceased. Blood plasma of light amber colour; no autolysin present; gives faint oxyhaemoglobin bands, 0·13 per cent. of haemoglobin being present in solution. No malarial parasites present in blood smear. The following observations were made at 37° C. with the patient's red blood cells suspended in 0·9 per cent. sodium chloride solution to which quinine bihydrochloride had been added:—

No. of experiment.	COMPOSITION OF MIXTURE OF RED BLOOD CELLS AND QUININE SOLUTION.			Haemolysis.
	2·5 % emulsion of red blood cells	0·9 % solution of sodium chloride	1·92 % solution of quinine bihydrochloride	
1	0·2 c.c.	1·2 c.c.	0·1 c.c.	Complete at end of ½ hour
2	0·5 c.c.	2·4 c.c.	0·1 c.c.	Complete at end of 1 hour
3	0·05 c.c.	4·6 c.c.	0·1 c.c.	Partial at end of 3 hours
4	1·7 c.c.	7·2 c.c.	0·1 c.c.	Unaffected at end of 3 hours

Condition of urine.—5th day (sample of twenty-four hours' urine). Sp. gr. 1·018; neutral to litmus paper; colour, brownish amber; contains a small amount of brownish deposit; after centrifugalisation the supernatant liquid gave no oxyhaemoglobin bands on spectroscopic examination; on acidifying and boiling, about one-fiftieth column of brownish white precipitate was obtained.

6th day. Characters as above, except that on acidifying and boiling slight turbidity appeared, but no precipitate.

The deposit in the urine consists of hyaline masses of casts filled with refractile granules of a faint brownish colour (Fig. 25, p. 53). Many of the casts or masses contain remains of nuclei, and sometimes vacuoles are also seen. No red cells or stromata. No brown amorphous deposit. No crystals.

Additional note. At the end of two and a half months another attack of blackwater occurred, terminating fatally at the end of three days. Patient was not seen by a medical man, and no further particulars of his illness were obtainable.

Blackwater Fever. Case 6.

Male, about thirty-nine years of age. Locomotive inspector. European.

Has lived in Rhodesia for fourteen years. First came to Nyasaland five months ago. Has had a good deal of malaria, both in Rhodesia and Nyasaland. Three years ago he had an attack of blackwater fever, and a second attack a year later. Never taken quinine unless ill. Four days ago he began to feel ill, but was able to continue at his work until yesterday, when he was decidedly worse. He thought he had malaria, and took three doses of quinine of ten grains each.

1st day. Was very unwell all to-day, and in the evening he noticed that his urine was red. T. 103° F.

2nd day. The urine continued red all day, and the patient's general condition was unchanged. Vomiting very troublesome, spleen enlarged and palpable. T. 98·2° F.

3rd day. Patient was rather better this morning. Urine became clear during the morning. Vomiting was still troublesome. T. 98° F.

4th day. Vomiting ceased, and patient felt much better. Urine amber coloured. T. 98·2° F.

Condition of blood.—3rd day. At 4 p.m. the oxalated plasma was found to be of a dark orange colour with no red tint, and gave in a column 18 mm. high faint bands of oxyhaemoglobin, equal to 0·16 per cent. of haemoglobin. No isolysin and no iso-agglutinin were present in the patient's blood plasma. No parasites and no pigmented leucocytes were found in the blood.

Condition of urine.—1st day. In the evening the urine was porter coloured.

2nd day. The urine remained porter coloured all day.

3rd day. This morning the urine was light red. Later in the day it cleared up, becoming dark amber coloured, sp. gr. 1·014, acid. There was a slight deposit on centrifugalising, consisting of granular casts, few red cells and epithelial cells. The supernatant fluid, when examined with the spectroscope, gave no bands of oxyhaemoglobin or methaemoglobin. There was a slight whitish precipitate on acidifying with acetic acid and boiling.

4th day. Urine was amber coloured, sp. gr. 1·016, no deposit on centrifugalising, and no precipitate on acidifying and boiling.

BLACKWATER FEVER. CASE 6a.

See history of preceding attack (Case 6). Has remained in good health since his last attack of blackwater fever about two months ago, with the exception of one attack of malaria which occurred about three weeks ago. During the past two months he has only taken an occasional ten-grain dose of quinine. Yesterday evening he did not feel very well, and, consequently, took ten grains of quinine and went to bed.

1st day. Patient got up this morning feeling better, and took another ten grains of quinine. Later in the day he began to feel very ill, and had to go to bed again. At 7-30 p.m. T. 103·4° F.. At 8 p.m. he passed blackwater. Vomiting very troublesome. At 11 p.m. T. 100·2° F.

2nd day. Condition unchanged to-day. Urine brownish coloured.

3rd day. Patient was rather exhausted. Vomiting troublesome. Skin and conjunctivae of a lemon-yellow colour. It was impossible to satisfactorily palpate the spleen owing to the rigidity with which the abdominal walls were held. Urine of brownish colour. T. 98·2° F.

4th day. Patient rather better, but vomiting was still severe. The urine had almost completely cleared during the morning.

5th day. The vomiting had stopped, and the urine was amber coloured.

Condition of blood. 3rd day. At 4-30 p.m. patient's oxalated plasma was of a pale orange colour with no red tint, containing 0·16 per cent. of haemoglobin. No autolysin was present in the plasma, and no parasites or pigmented leucocytes were found in the blood.

Condition of urine

Date and time.	Amount.	Sp. gr.	Reaction.	Colour.	Appearance and amount of deposit.	Spectroscopic examination.	Amount of haemoglobin passed.
1st day (evening)	—	—	—	Porter coloured	—	—	—
2nd day	170 c.c.	1·015	Acid	Brown	Deposit on centrifugalising small in amount, consisting of granular casts and débris, red cells and epithelial cells. Also a few crystals of triple phosphates. (There were about 50 red cells to the field).	No bands in the supernatant fluid after centrifugalising	Light brownish precipitate on acidifying and boiling, equalling less than 0·8 % of haemoglobin.
3rd day up till 4.30 p.m.	57 c.c.	1·015	”	”	” ”	”	Slight precipitate on acidifying and boiling.
” 4.40 ”	28·5 c.c.	1·015	”	”	” ”	”	”
4th day, 8.0 a.m.	228 c.c.	1·015	”	”	” ”	”	”
” 9.0 ”	28 c.c.	1·015	”	”	” ”	”	”
5th day	—	1·017	”	Amber coloured	Very slight deposit as above.	”	Faint cloud on acidifying and boiling.

Subsequently the urine was amber coloured and normal.

BLACKWATER FEVER. CASE 7.

Male, thirty-two years of age. Clerk. Eurasian.

Has been in Nyasaland for six years. One month after coming into the country had an attack of malaria lasting ten days. Nine months later had blackwater fever. Suffered from malaise for about a fortnight, gradually getting worse. Then observed that his urine was of a reddish-black colour, resembling stout. Vomited severely, and had general aching pains. Complexion became sallow, was very weak. At end of three days urine became normal. Patient was well again at end of ten days. Two and a half years ago had dysentery. Six months ago had malaria, his temperature at the time reaching 105° F.

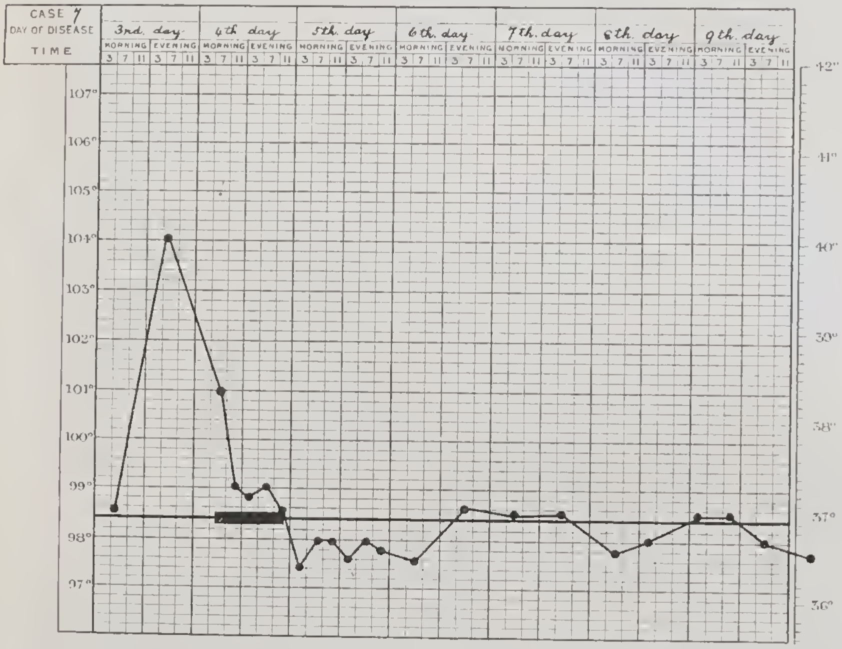

FIG. 73. Blackwater Fever, Case 7. Temperature Chart.

1st day of illness. Felt ill, and went to bed in the afternoon.

2nd day. Did not feel very well in the morning, but did not leave off work. At four o'clock in the afternoon had an attack of ague and went to bed again, shivering and sweating very much. Took ten grains of quinine in five-grain doses.

3rd day. In the morning patient's temperature was not raised. Felt fairly well, except for an empty feeling at the stomach and a sense of weakness. In the afternoon vomited several times, and in the evening had a temperature of 104° F. Noticed that his urine was somewhat high coloured, but this he did not think unusual. Took fifteen grains of quinine, in five-grain doses, during the day.

4th day. Noticed that his colour was sallow, and on passing his urine observed that it resembled stout, being dark coloured. Has pain in the hypogastric region. Has had pain in loins for last three days, but no general pains, as was the case during the previous attack of blackwater fever. No constitutional disturbance. Temperature normal. The viscera appear healthy. No recognisable enlargement of the spleen. Is anaemic and sallow.

5th day. Urine amber coloured. General condition good Appetite fair. Temperature normal.

Recovery was uninterrupted, no relapse occurring.

Condition of blood. 4th day, 12-30 p.m. Blood plasma of dark orange colour, no reddish tint being observable; gives faint oxy-haemoglobin bands in a column eighteen millimetres high, 0·13 per cent. of dissolved haemoglobin being present; no autolysin present. The following observations were made at 37° C. with the patient's red blood cells suspended in 0·9 per cent. sodium chloride solution, to which quinine bihydrochloride had been added.—

No. of experiment.	COMPOSITION OF MIXTURE OF RED BLOOD CELLS AND QUININE SOLUTION.			Haemolysis.
	2·5 % emulsion of red blood cells.	0·9 % solution of sodium chloride.	1·92 % solution of quinine bihydrochloride.	
1	0·2 c.c.	1·2 c.c.	0·1 c.c.	Complete at end of 3
2	0·5 c.c.	2·4 c.c.	0·1 c.c.	Complete at end of 1
3	1·05 c.c.	4·6 c.c.	0·1 c.c.	Partial at end of 3 hour
4	1·7 c.c.	7·2 c.c.	0·1 c.c.	Unaffected at end of 3

7th day. Blood smear negative.

8th day. Blood plasma of light orange colour, contains abo 0·06 per cent. of dissolved haemoglobin.

Condition of urine. The characters of the urine are given in the following table:—

Date.		Amount.	Specific gravity.	Reaction.	Colour.	Amount and appearance of deposit.	Spectroscopic examination
h day	9.30 a.m.	650 c.c.	1·030	Slightly alkaline	Porter coloured	$\frac{1}{6}$ col. dark brown	No oxyhaemoglobin bands
,,	4.15 ,,	190 ,,	—	Alkaline	,,	$\frac{1}{10}$ col. dark brown	,, ,,
,,	9.30 p.m.	140 ,,	—	,,	Brown amber	Less than above	,, ,,
th day.	1.40 a.m.	200 ,,	1·017	Acid	Dark amber	Very slight	
,,	7.20 ,,	150 ,,	1·027	,,	Amber	,,	
,,	1.30 p.m.	190 ,,	1·015	,,	Light amber	,,	
,,	6.0 ,,	130 ,,	1·010	,,	,,	,,	

The deposit present in the urine on the fourth day consisted of granular masses and casts, the granules being fine, apparently refractile, and brown in colour, held together by hyaline material. A few renal epithelial cells were seen. No red blood cells or stromata. No crystalline deposit.

On centrifugalising the first specimen of urine the supernatant liquid was found to be quite clear, of a dark brown colour without any reddish tint, and on acidifying and boiling gave about one-twentieth column of a light brown flocculent precipitate. The amount of dissolved haemoglobin originally present was therefore small, presumably not exceeding 0·15 per cent. The total amount of haemoglobin in the urine apparently did not exceed that contained in $980 \times 0·0015 = 1·5$ c.cm. of healthy red cells.

BLACKWATER FEVER. CASE 7a.

Male, thirty-two years of age. Clerk. Eurasian.

(For account of previous attack of blackwater fever, two months ago, see preceding report.)

Was well up to ten days ago, when he had febrile disturbance, attributed to malaria, and commenced taking ten grains of quinine every day. As his temperature did not become normal he increased the amount, five days ago, to fifteen grains a day. His temperature then rose to 104° F., the rise being accompanied by a rigor and cramp of the stomach, and followed by profuse sweating. Patient expected that this would be the forerunner of an attack of blackwater, but his urine remained clear and amber coloured. Four days ago, and again three days ago, fifteen grains of quinine, in two and a half grain doses, were taken. His temperature, however, still remained persistently high. Two days ago he took twelve and a half grains of quinine; yesterday he took fifteen grains. During the last three days drank little fluid and passed little urine.

1st day. During the night patient's temperature rose to 105° F. At 5 a.m. passed 350 c.cm. of dark red urine. Has considerable constitutional disturbance. Skin and conjunctivae of a yellow colour. Sweating profusely. Spleen enlarged, projecting about one and a quarter inches below the costal margin at the end of inspiration. From this day onwards no more quinine was taken by patient.

2nd day. Temperature remains low. General condition good. Somewhat depressed. Vomiting of green and yellow bile-stained mucus, troublesome. Pulse good, feels fairly comfortable. Skin still fairly yellow. Urine amber coloured; total amount 18 c.cm.

3rd day. Vomiting continues, otherwise general condition good. Pulse not feeble. Began to perspire when a hot pack was applied, his temperature rising to 102°2F. Total urine 21 c.cm. Received intravenously at 4 p.m. one litre of 0·9 per cent. solution of sodium chloride. Slight rigor one hour later. General condition showed considerable improvement after saline injection. Vomiting ceased. Retained two pints of fluid drink.

4th day. General condition good. Received subcutaneously 800 c.cm. of saline solution. Felt much better after injection. Total urine 11 c.cm.

5th day. Had a good night. General condition remarkably good. Vomited greenish bile on one occasion only. Takes a little semi-fluid food. Total urine 17 c.cm.

6th day. General condition good. Slight vomiting once to-day. Takes and retains a little food. Had slight dyspnoea twice yesterday. Total urine 35 c.cm.

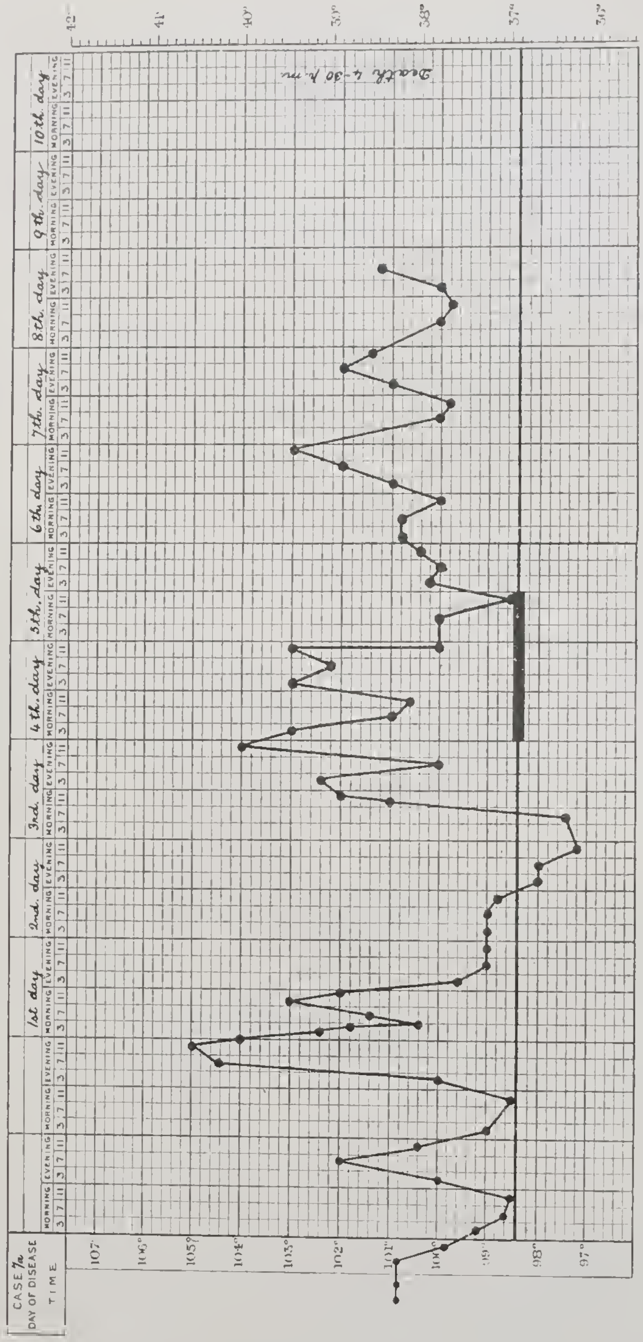

FIG. 74. Blackwater Fever, Case 7a. Temperature Chart

8th day. General condition has remained good until to-day. Is now lethargic and feeble. Has occasional dyspnoea. Had Cheyne Stokes respiration for a short time last night. Vomiting recommenced to-day. Vomited three times this morning. In the evening dyspnoea increased, especially after slight movement in bed, patient became restless and his extremities cold. Slight anasarca of hands and face. A few fine râles at lower borders of lungs, breath sounds loud and harsh generally. Total urine 41 c.cm.

9th day. Slept last night after administration of morphia. Much improved again this morning. Total urine 23 c.cm.

10th day. Except for dyspnoea is fairly comfortable. Died suddenly at 5 p.m. from cardiac failure. Total urine 43 c.cm.

Condition of blood.—1st day, 10 a.m. Blood plasma of deep orange colour, no distinct reddish tint being recognisable with certainty; contains 0·74 per cent. of dissolved haemoglobin; no autolysin present. No malarial parasites present in blood smear. The following observations were made at 37° C. with the patient's red blood cells suspended in 0·9 per cent. sodium chloride solution to which quinine bihydrochloride had been added:

No. of experiment.	COMPOSITION OF MIXTURE OF RED BLOOD CELLS AND QUININE SOLUTION.			Haemolysis.
	2·5 % emulsion of red blood cells	0·9 % solution of sodium chloride	1·92 % solution of quinine bihydrochloride	
1	0·5 c.c.	2·4 c.c.	0·1 c.c.	Complete in 1 hour.
2	0·65 c.c.	3·0 c.c.	0·1 c.c.	Complete in 2 hours.
3	0·8 c.c.	3·6 c.c.	0·1 c.c.	Marked in 3 hours.
4	1·05 c.c.	4·6 c.c.	0·1 c.c.	Trace in 3 hours.
5	1·3 c.c.	5·6 c.c.	0·1 c.c.	Trace in 3 hours.

Condition of urine. The characters of the urine are given in the following table:

Date.	Amount.	Specific gravity.	Reaction.	Colour.	Amount and appearance of deposit.	Spectroscopic examination.
t day, 5.0 a.m.	350 c.c.	1·032	Alkaline	Dark porter coloured	$\frac{1}{30}$ col. brown	Oxyhaemoglobin, no methaemoglobin
,, 2.0 p.m.	45 ,,	1·028	Neutral	,,	$\frac{1}{15}$ col. brownish black	Faint O_2 Hb. bands, no methaemoglobin bands
,, 6.30 ,,	30 ,,	1·020	,,	,,	$\frac{1}{15}$,, ,,	Same as preceding
,, 10.0 ,,	12 ,,	1·022	Faintly alkaline	Dark red black	$\frac{1}{30}$ col. ,,	Strong O_2 Hb. and methaemoglobin bands
ıd day, 9.30 a.m.	15 ,,	1·010	Alkaline	Port-wine coloured	$\frac{1}{30}$,, ,,	Oxyhaemoglobin, no methaemoglobin
,, 1.0 p.m.	1·5 ,,	—	,,	Brown	Very Slight	No haemoglobin
,, 2.0 ,,	1·5 ,,	—	,,	Yellow	,,	,,
d day, 12.30 a.m.	3 ,,	—	,,	,,	,,	,,
,, 3.15 p.m.	15 ,,	—	,,	Brownish amber	,,	,,
,, 7.0 ,,	3 ,,	—	,,	,,	,,	,,
h day, 4.0 a.m.	2·5 ,,	—	,,	Light yellow	,,	,,
,, 10.0 ,,	4 ,,	—	,,	,,	,,	,,
,, 7.30 p.m.	4·5 ,,	—	,,	,,	,,	,,
h day, 7.0 a.m.	6 ,,	1·012	,,	Clear yellow	,,	,,
,, 11.30 a.m.	5 ,,	—	,,	,,	,,	,,
,, 3.0 p.m.	3 ,,	—	,,	,,	,,	,,
,, 6.30 ,,	3 ,,	—	,,	,,	,,	,,
ı day, 8.0 a.m.	18 ,,	1·010	,,	,,	,,	,,
,, 12.30 ,,	6 ,,	—	,,	,,	,,	,,
,, 5.30 ,,	7 ,,	—	,,	,,	,,	,,
,, 7.30 ,,	2 ,,	—	Faintly alkaline	,,	,,	,,
ı day, 2.0 a.m.	15 ,,	1·015	Alkaline	Light yellow	,,	,,
,, 5.0 a.m.	6 ,,	,,	,,	,,	,,	,,
,, 11.30 ,,	8 ,,	,,	Faintly alkaline	,,	,,	,,
,, 3.30 p.m.	8 ,,	,,	Alkaline	,,	,,	,,
,, 9.30 ,,	8 ,,	,,	,,	,,	,,	,,
,, 11.5 ,,	3 ,,	—	,,	,,	,,	,,
ı day, 4.0 a.m.	12 ,,	1·014	,,	,,	,,	,,
,, 12.30 p.m.	9 ,,	,,	,,	,,	,,	,,
,, 3.30 ,,	7 ,,	—	,,	,,	,,	,,
,, 8.30 ,,	7 ,,	1·015	,,	,,	,,	,,
,, 10.0 ,,	6 ,,	—	,,	,,	,,	,,
day, 2.30 p.m. (by catheter)	13 ,,	1·018	Faintly alkaline	,,	,,	,,
,, 5.30 p.m.	3·5 ,,	1·015	Alkaline	,,	,,	,,
,, 7.0 ,,	7 ,,	,,	,,	,,	,,	,,
day, 10.0 a.m. (by catheter)	43 ,,	,,	Faintly alkaline	,,	,,	,,

The total amount of urine passed on the first day was 397 c.c.; on the succeeding nine days the average daily amount was 28·5 c.c.

The deposit in the urine on the first day consisted of yellowish brown granules, forming a precipitate, or arranged in masses or in well defined casts (Fig. 29, p. 100); the individual granules reaching up to 4·5μ in diameter; unaltered red cells in small numbers (not present in the first specimen of urine); squamous epithelial cells; and (in the first specimen only) triple phosphate crystals. Subsequently the amount of deposit became very small and consisted, even in the last specimen, of red cells; coarse granular casts surrounded by renal tubule cells (Fig. 32, p. 104); granular débris; and renal cells, isolated or in groups.

The first, third, fourth and fifth specimens of urine gave (after centrifugalisation) the following percentages of haemoglobin, determined by means of a comparison spectroscope: 0·41 per cent.; 1·1 per cent.; 1·2 per cent.; 0·8 per cent. These estimations of the oxyhaemoglobin actually present do not represent the amounts actually passing into the urine in the kidney, since much of the haemoglobin had been decomposed. On comparing the chocolate brown precipitate obtained with that yielded by boiling a mixture of blood and urine, it was found that the samples of urine were matched as follows:—1st, with 1·8 per cent. of haemoglobin; 3rd, with 2·5 per cent. of haemoglobin; 4th, with 3·5 per cent. of haemoglobin; 5th, with 3·5 per cent. of haemoglobin. The total haemoglobin lost by the kidneys on the first day did not therefore exceed 6·4 + 0·99 + 0·75 + 0·42 + 0·52 = 9·06 g. of wet red blood cells. The yellow coloured urine subsequently passed contained $\frac{1}{4}$ to $\frac{2}{3}$ column of coagulable proteid of a slightly brownish white colour.

Post-mortem examination.—Body well nourished. Skin no longer sallow. Subcutaneous tissue oedematous generally.

Peritoneal cavity contained about 900 c.cm. of clear yellowish fluid. Spleen much enlarged: 6in. long, 4in. broad, and 2in. thick. Spleen smear showed phagocytosis of red blood cells, but no malarial parasites or pigment. Liver somewhat enlarged, milky opacity on surface, some congestion. Kidneys markedly enlarged, measuring slightly more than 5in. in length, 3in. in breadth, and 1$\frac{1}{4}$in. in thickness; capsule stripped readily; cortex not markedly congested.

brown in colour; pyramids swollen, dark almost black, with a striated aspect; mucous membrane of pelvis showed punctate ecchymoses; superficial ecchymosis on surface of left kidney, about half an inch in diameter. Intestines distended with gas. Stomach empty; mucous membrane congested.

Recent pleural adhesions on right side; older adhesions on left side; pleural cavities obliterated. Lungs emphysematous; scars on surface of upper lobe near apex; numerous calcified and caseous nodules. Bronchial glands enlarged, not tubercular. Pericardium contained about 10 c.cm. of clear yellowish fluid; valves of heart healthy; myocardium somewhat pale; cavities of heart small.

Bladder normal in aspect. Suprarenals unaltered. Pancreas natural in appearance.

Microscopical examination. The kidneys presented marked distension of the tubules and malpighian capsules. In some of the tubules solid material consisting of coarse brown granules, rarely exceeding 5μ in diameter, together with irregular masses formed by the aggregation of these granules, and sometimes also free epithelial cells and finely granular or flocculent material, were found partly filling the lumen of the tubule; further details are given on pp. 107-118. The interstitial tissue was free from haemorrhage and showed no cell infiltration or increase of connective tissue. The blood vessels contained no malarial pigment.

The liver showed slight fatty change and some congestion, but no cirrhosis. There was no pigment in the liver cells and no malarial pigment in the blood vessels of the liver.

The spleen exhibited no malarial pigment.

BLACKWATER FEVER. CASE 8.

Male, about thirty-eight years of age. Joiner. Chinaman.

Has lived in Delagoa Bay and Chinde for ten years. First came to Nyasaland two years ago. During this time he has had a considerable amount of fever, but has never previously had blackwater fever. Has rarely taken quinine except after an attack of fever.

He commenced to be ill with fever three days ago. Stayed in bed and took fifteen grains of quinine on this and on each of the two following days.

4th day. At 2 p.m. he had a rigor, T. 104·4° F., and shortly afterwards passed porter coloured urine. Had slight vomiting.

5th day. Patient felt better this morning. The vomiting had ceased, and the urine during the morning became almost clear. About noon the urine became red again. At 2-30 p.m. T. 102° F. Vomiting was troublesome. The spleen was enlarged and easily palpable.

6th day. Patient better to-day. Urine amber coloured. At 10 a.m. three grains of quinine bihydrochloride were given hypodermically, and at 2 p.m. two and a half grains were given by the mouth.

7th day. About noon to-day there was a slight relapse, the urine becoming claret coloured. One grain of quinine was given three times to-day.

8th day to 13th day. Quinine bihydrochloride was administered thrice daily in two grain doses. The urine remained amber coloured during this period.

14th day. Three grains of quinine bihydrochloride were given thrice daily.

15th day. Patient remained well, no further relapse; the urine continued to be amber coloured.

Condition of blood. 5th day. At 1-45 p.m. patient's oxalated plasma was of a deep orange colour, with no red tint, containing 0·13 per cent. of haemoglobin. No bands of methaemoglobin were seen on examining a column of the plasma 18 mm. deep. No autolysin was present in the plasma, and no parasites or pigmented leucocytes were found in the blood. The following observations were made at 37° C. with the patient's red blood cells suspended in 0·9 per cent. sodium chloride solution to which quinine bihydrochloride had been added :

No. of experiment.	Composition of Mixture of Red Blood Cells and Quinine Solution.			Haemolysis.
	2·5 % emulsion of red blood cells	0·9 % solution of sodium chloride	1·92 % solution of quinine bihydrochloride	
1	0·4 c.c.	2·0 c.c.	0·1 c.c.	Complete at end of 3 hours
2	0·5 c.c.	2·4 c.c.	0·1 c.c.	Complete at end of 3 hours
3	0·65 c.c.	3·0 c.c.	0·1 c.c.	Complete at end of 3 hours
4	0·8 c.c.	3·6 c.c.	0·1 c.c.	Complete at end of 3 hours
5	1·05 c.c.	4·6 c.c.	0·1 c.c.	Almost complete at end of 3 hours
6	1·3 c.c.	5·6 c.c.	0·1 c.c.	Slight at end of 3 hours
7	1·5 c.c.	6·4 c.c.	0·1 c.c.	Slight at end of 3 hours
8	1·7 c.c.	7·2 c.c.	0·1 c.c.	Trace at end of 3 hours

6th day. At 10 a.m. patient's oxalated plasma was of a deep orange colour, with no red tint, containing 0·07 per cent. of haemoglobin in solution. No parasites or pigmented leucocytes were found in blood smears. The following observations were made at 37° C. with the patient's red blood cells suspended in 0·9 per cent. sodium chloride solution to which quinine bihydrochloride had been added :—

No. of experiment.	Composition of Mixture of Red Blood Cells and Quinine Solution.			Haemolysis.
	2·5 % emulsion of red blood cells	0·9 % solution of sodium chloride	1·92 % solution of quinine bihydrochloride	
1	0·5 c.c.	2·4 c.c.	0·1 c.c.	Complete at end of 3 hours
2	0·65 c.c.	3·0 c.c.	0·1 c.c.	Complete at end of 3 hours
3	0·8 c.c.	3·6 c.c.	0·1 c.c.	Complete at end of 3 hours
4	1·05 c.c.	4·6 c.c.	0·1 c.c.	Slight at end of 3 hours
5	1·3 c.c.	5·6 c.c.	0·1 c.c.	Trace at end of 3 hours
6	1·5 c.c.	6·4 c.c.	0·1 c.c.	Nil at end of 3 hours

Condition of urine.

Date and time.	Amount.	Sp. gr.	Reaction.	Colour.	Appearance and amount of deposit.	Spectroscopic examination.	Amount of haemoglobin.
4th day	—	—	—	Porter coloured	—	—	—
5th day, noon	—	—	Acid	Port wine coloured	Deposit on centrifugalising slight, consisting of granular casts and few renal epithelial and red cells	Oxyhaemoglobin and methaemoglobin bands, both distinct	0·2 %
,, 1.45 p.m.	—	—	,,		,, ,,	,,	0·1 %
,, 2.50 ,,	114 c.c.	1·012	,,	Dark brown	,, ,,	,,	0·15 % (corresponding to 0·17 gr. of haemoglobin)
,, 5.0 ,,	170 c.c.	1·008	,,	,,	,, ,,	No bands	Trace of precipitate on acidifying and boiling
,, 7.0 ,,	114 c.c.	1·008	,,	Dark amber coloured	Very slight deposit	,,	,,
,, 10.0 ,,	114 c.c.	1·008	,,	,,	,,	,,	,,
,, 11.30 ,,	170 c.c.	1·014	,,	,,	,,	,,	,,
6th day, 6.0 a.m.	285 c.c.	1·014	,,	,,	Very slight deposit as above	,,	,,
,, 9.0 ,,	114 c.c.	1·020	,,	,,	,,	,,	,,
,, 10.45 ,,	140 c.c.	1·022	,,	,,	,,	,,	,,
,, 11.30 ,,	85 c.c.	1·016	,,	,,	,,	,,	,,
,, noon	114 c.c.	1·008	,,	Pale amber coloured	,,	,,	,,
,, 1.30 p.m.	285 c.c.	1·005	,,	,,	,,	,,	No precipitate on acidifying and boiling
7th day, noon	—	—	—	Red			

The next day the urine was amber coloured and normal and the subsequent specimens were all normal.

BLACKWATER FEVER. CASE 9.

Male, thirty-eight years of age. Contractor. European.

Has been in the Tropics for fifteen years. Has had dysentery twice, the last attack two years ago. Has had 'fever' (malaria) many times. Has suffered from rheumatic pains. Has not had blackwater fever before present attack.

Has not been feeling well for some weeks. Appetite has been poor. Has been inclined to doze in daytime. For the last three weeks has had 'fever,' for which he has been taking a Durban patent 'cure.' His urine has been of a dark brownish colour for last six weeks. Yesterday took nine grains of quinine in tabloid form.

1st day. Thinks he passed blackwater in the morning when at stool, but regarded the colour of his urine as due to bile. Took nine grains of quinine during the day. Stayed indoors and had a rigor at mid-day, when he passed more blackwater, which he still regarded as due to bile. At 6 p.m. his urine was porter coloured, about 170 c.cm. being passed. Temperature at this time 104·2 F.

2nd day. Constitutional disturbance continues. Urine still remains porter coloured. Vomited about twenty times during evening and night.

3rd day. Urine still red. Lighter coloured in evening.

4th day. Urine at first red, later became amber coloured.

5th day. During the 2nd, 3rd, 4th and 5th days only about 600 c.c. of urine was passed, together with a small but inconsiderable amount in the stools, and suppression of urine was feared. Cardiac action feeble.

6th day. Passing urine more freely.

7th day. Continues to pass urine freely. Is very pale and anaemic, but not wasted. Not sallow. Pulse hard and sharp, of high tension. Cardiac dulness abolished. Apex beat cannot be felt, but the first sound is at a maximum three and a half inches from midsternal line and about half an inch inside the nipple line. No adventitious sounds. Second sound at base over aortic orifice accentuated. Liver not palpable. Liver dulness not increased. Spleen extends at the end of inspiration, about four inches beyond the costal margin. Splenic dulness considerably increased. During the attack of blackwater has had pain in loins, not descending into testes or thighs.

Condition of blood. 8th day. On pricking patient's finger the blood obtained was watery in aspect. The red cells were relatively small in amount, and rapidly sank in the (oxalated) plasma. Plasma light yellow, gives a faint oxyhaemoglobin spectrum, but no methaemoglobin bands, in a layer eighteen millimetres high; amount of dissolved haemoglobin is less than 0·06 per cent. On testing for autolysin (eight hours after withdrawal of blood) complete haemolysis was obtained in two and a half hours (Table 29); bacteria were, however, present at the time. (On testing blood plasma for autolysin three and a half months later a negative result was obtained.)

No malarial parasites found in blood smear. The following observations were made at 37° C. with the patient's red blood cells, suspended in 0·9 per cent. sodium chloride solution to which quinine bihydrochloride had been added:—

No. of experiment.	Composition of Mixture of Red Blood Cells and Quinine Solution.			Haemolysis.
	2·5 % emulsion of red blood cells	0·9 % solution of sodium chloride	1·92 % solution of quinine bihydrochloride	
1	0·65 c.c.	3·0 c.c.	0·1 c.c.	Complete in 1 hour
2	0·8 c.c.	3·6 c.c.	0·1 c.c.	Complete in 2½ hours
3	1·05 c.c.	4·6 c.c.	0·1 c.c.	Trace at end of 3 hours

Condition of urine.—8th day. Yellow in colour. On adding a drop of acetic acid and boiling becomes cloudy, and an inconsiderable amount (about a $\frac{1}{40}$ column) of whitish precipitate is formed. Contains a small amount of suspended matter consisting chiefly of pus cells, together with squamous epithelial cells and a few granular masses or casts of a faint brownish tint; no red cells or stromata; no granular pigment.

BLACKWATER FEVER. CASE 10

Male, twenty-six years of age. Engineer. European

Has lived in Nyasaland for two years, and during this time he has had a considerable amount of fever. He has taken very little quinine during the last twelve months. About three weeks ago he had a severe attack of fever which lasted four or five days. A few days later he was run over by a trolley, and has been confined to bed more or less since. He was unable to sleep and felt very ill. Three days ago he felt particularly unwell and had a sore throat. The next day his temperature was 102° F., and he took seven and a half grains of quinine. Yesterday his temperature was 103° F., and he took another seven and a half grains of quinine.

1st day. He felt better this morning and took ten grains of quinine and was able to get up. At noon he had five grains of quinine and took a short walk. At 4 p.m. he suddenly felt very ill. He had a severe rigor. T. 105° F. At 5 p.m. he passed blackwater. Had troublesome vomiting and flatulency all night.

2nd day. Vomiting had ceased and patient felt better. Spleen enlarged and easily palpable, extending one and a half inches below the costal margin.

3rd day. Patient was better to-day; the urine was not so darkly coloured.

4th day. About noon there was a relapse. T. 103° F.

5th day. Patient admitted to hospital this evening. Blackwater continued. T. 103° F.

6th day. The urine was amber coloured this morning. About noon it became red again, but cleared up in the afternoon. There was another relapse late in the evening.

7th day. The urine remained coloured all day; in the evening T. 103·6° F.

8th day. Urine still contained haemoglobin. Quinine bihydrochloride was given in solution in two and a half grain doses at 6 a.m. and 9 a.m. Later in the day the urine became amber coloured. T. 102·4° F.

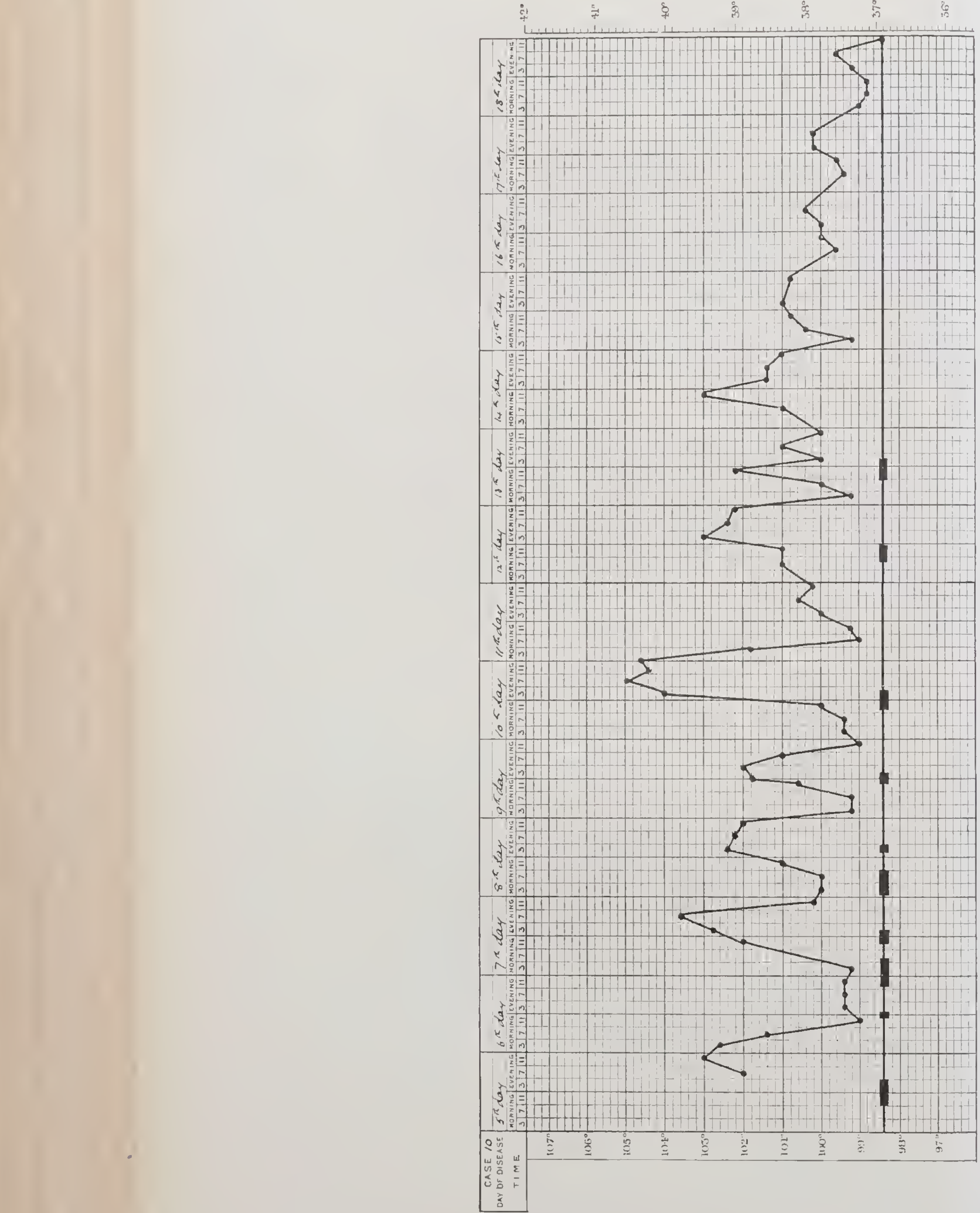

FIG. 75. Blackwater Fever, Case 10. Temperature Chart.

9th day. The urine was coloured again this morning, but cleared up in the evening. Quinine bihydrochloride was given as before in two doses of two and a half grains each. T. 102° F.

10th day. The urine, which was clear this morning, became deeply red coloured about mid-day. Quinine bihydrochloride was given in solution in three doses of three and three quarter grains each. T. 104° F. Patient appeared to be in fairly good condition.

11th day. The urine remained clear all day. Quinine bihydrochloride was given in three doses of five grains each. T. 100·6° F.

12th day. There was another slight relapse this morning. Quinine bihydrochloride was given again in three doses of five grains each. T. 103° F.

13th day. The urine, which had become amber coloured yesterday afternoon, was again red about noon to-day. Quinine bihydrochloride was given in three doses of five grains each. T. 102° F.

14th day. There was a slight brownish coloration of the urine about noon to-day, but no detectable amount of haemoglobin. No quinine was given to-day. T. 103° F.

15th day. Urine normal all day. No quinine given. T 101° F.

16th day. Urine normal. No quinine given. T. 100·4° F.

Patient remained well and had no further relapses. About fourteen days later small doses of quinine were commenced and the quantity gradually increased until fifteen grains were given daily. No further relapses occurred

Condition of blood.—2nd day. At 11 a.m. patient's oxalated plasma was of a deep orange colour with a distinct reddish tint, and gave marked bands of oxyhaemoglobin in a column 18 mm. high, representing 0·85 per cent. of dissolved haemoglobin. Faint bands of methaemoglobin were visible. No autolysin was present in the serum, and no parasites or pigmented leucocytes were found in the blood. The following observations were made at 37° C. with the patient's red blood cells suspended in 0·9 per cent. sodium chloride solution, to which quinine bihydrochloride had been added.

No. of experiment.	Composition of Mixture of Red Blood Cells and Quinine Solution.			Haemolysis.
	2·5 % emulsion of red blood cells	0·9 % solution of sodium chloride	1·92 % solution of quinine bihydrochloride	
1	0·4 c.c.	2·0 c.c.	0·1 c.c.	Complete at end of 1 hour
2	0·5 c.c.	2·4 c.c.	0·1 c.c.	Complete at end of 1 hour
3	0·65 c.c.	3·0 c.c.	0·1 c.c.	Complete at end of 2 hours
4	0·8 c.c.	3·6 c.c.	0·1 c.c.	Complete at end of 2½ hours
5	1·05 c.c.	4·6 c.c.	0·1 c.c.	Trace at end of 3 hours
6	1·3 c.c.	5·6 c.c.	0·1 c.c.	Nil at end of 3 hours

3rd day. At 10-30 a.m. patient's oxalated plasma was deep orange, with no red tint, containing 0·09 per cent. of haemoglobin in solution. The bands of methaemoglobin were visible but faint. No parasites or pigmented leucocytes were found in the blood.

5th day. At 11 a.m. patient's oxalated plasma was a deep orange colour and gave, in a column 18 mm. high, distinct oxyhaemoglobin bands, the amount of dissolved haemoglobin being 0·3 per cent. The methaemoglobin bands were just visible. No autolysin present in the plasma. The appearance of the blood when withdrawn was distinctly watery. No parasites or pigmented leucocytes were found in the blood. The following observations were made at 37° C. with the patient's red blood cells suspended in 0·9 per cent. sodium chloride solution, to which quinine bihydrochloride had been added.

No. of experiment.	Composition of Mixture of Red Blood Cells and Quinine Solution.			Haemolysis.
	2·5 % emulsion of red blood cells	0·9 % solution of sodium chloride	1·92 % solution of quinine bihydrochloride	
1	0·5 c.c.	2·4 c.c.	0·1 c.c.	Complete at end of 1 hour
2	0·65 c.c.	3·0 c.c.	0·1 c.c.	Complete at end of 2 hours
3	0·8 c.c.	3·6 c.c.	0·1 c.c.	Very marked at end of 3 hours
4	1·05 c.c.	4·6 c.c.	0·1 c.c.	Trace at end of 3 hours

6th day. At 11 p.m. patient's oxalated plasma was of a dark orange colour with no red tint; it gave distinct oxyhaemoglobin bands, 0·2 per cent. of haemoglobin being present in solution. No parasites or pigmented leucocytes were found in the blood.

Condition of urine

Date and time.	Amount.	Sp. gr.	Reaction.	Colour.	Appearance and amount of deposit.	Spectroscopic examination.	Amount of haemoglobin.
1st day, 6.30 p.m.	12 c.c.	1·022	Acid	Dark red	Considerable amount of deposit consisting entirely of granular casts and débris. No red cells.	Marked haemoglobin and methaemoglobin bands.	0·6 % (corresponding to 0·1 gr. of haemoglobin). Total haemoglobin passed during day equals 0·1 gr.
2nd day, 11.0 a.m.	—	—	„	Port wine red	Deposit $\frac{1}{80}$ column consisting of granular casts and squamous epithelium. No red cells.	„	1·1 %
„ 3.30 p.m	205 c.c.	1·024	„	Porter coloured	Deposit considerable, consisting of granular casts and debris and epithelial cells. Very few red cells (about 1 in a field).	„	0·65 % (corresponding to 1·3 gr. of haemoglobin).
„ 4.30 „	130 c.c.	1·020	„	„	„ „	„	0·85 % (corresponding to 1·1 gr. of haemoglobin).
„ 6.30 „	95 c.c.	1·020	„	„	„ „	„	1·1 % (corresponding to 1·1 gr. of haemobgloin).
„ 8.0 „	200 c.c.	1·016	„	„	„ „	„	1·1 % (corresponding to 2·2 gr. of haemoglobin). Total amount of haemoglobin passed during day equals 5·7 gr.
3rd day, 10.30 a.m.	—	—	—	Brown	Slight deposit consisting mainly of granular casts.	Bands of oxyhaemoglobin and methaemoglobin, both faint	0·1 %
„ 1.0 p.m.	120 c.c.	1·018	Acid	„	„ „	„	—
„ 7.0 p.m.	—	—	—	„	„ „	„	—
4th day, 10.0 a.m.	—	1·024	Acid	„	Considerable deposit on centrifugalising consisting almost entirely of granular casts; very few red cells.	„	—
„ noon	—	1·022	„	Porter coloured	„ „	Strong bands of oxyhaemoglobin and methaemoglobin.	0·8 %
„ 2.0 p.m.	—	1·016	„	Port wine colour	„ „	Bands of oxyhaemoglobin and methaemoglobin just visible.	Large chocolate coloured precipitate on acidifying and boiling equalling ½ column.

Date and time.	Amount.	Sp. gr.	Reaction.	Colour.	Appearance and amount of deposit.	Spectroscopic examination.	Amount of haemoglobin.
4th day 4.0 p.m.	—	1·012	Acid	Light brown	Slight deposit as above.	Bands of oxyhaemoglobin and methaemoglobin just visible.	Light brown precipitate on acidifying and boiling equalling $\frac{1}{10}$ column.
,, 5.30 ,,	—	1·003	,,	Amber	No deposit.	,,	Very faint cloud on acidifying and boiling.
5th day, 9.30 a.m.	—	1·020	,,	Porter coloured	Deposit slight consisting of granular casts and epithelial cells.	Bands of oxyhaemoglobin and methaemoglobin well marked.	0·75 %; large chocolate coloured precipitate on acidifying and boiling equalling $\frac{1}{2}$ column.
,, 11.0 ,,	—	1·014	,,	Dark red	,, ,,	,,	1·6 %; chocolate coloured precipitate on acidifying and boiling equalling $\frac{1}{3}$ column
,, 2.30 p.m.	200 c.c.	1·010	,,	Reddish brown	,, ,,	Faint bands of oxyhaemoglobin and methaemoglobin.	0·05 %; chocolate coloured precipitate on acidifying and boiling equals $\frac{1}{5}$ col.
,, 4.30 ,,	300 c.c.	1·002	,,	Amber	No deposit.	No bands	Slight white precipitate on acidifying and boiling.
,, 7.0 ,,	300 c.c.	1·005	,,	,,	,,	,,	,,
,, 9.30 ,,	200 c.c.	1·010	,,	,,	,,	Bands of oxyhaemoglobin just visible.	,,
,, 11.30 ,,	—	1·004	,,	,,	,,	,,	,,
6th day, 3.0 a.m.	—	1·011	,,	,,	,,	No bands	No precipitate on acidifying and boiling.
,, 6.15 ,,	—	1·012	,,	,,	,,	,,	,,
,, noon	—	1·020	,,	Brown	Slight deposit consisting of granular casts.	Faint bands of oxyhaemoglobin.	Brownish precipitate on acidifying and boiling equals $\frac{1}{15}$ column.
,, 2.30 p.m.	—	1·010	,,	Dark amber	,, ,,	No bands	Very slight precipitate on acidifying and boiling.
,, 10.30 ,,	—	1·012	Neutral	Light red	Whitish deposit on centrifugalising about $\frac{1}{5}$ column consisting of granular casts and triple phosphate crystals.	Well marked bands of oxyhaemoglobin.	0·36 %; brownish precipitate on acidifying and boiling equals $\frac{1}{5}$ column.

Date and time.	Amount.	Sp. gr.	Reaction.	Colour.	Appearance and amount of deposit.	Spectroscopic examination.	Amount of haemoglobin.
7th day 7.30 a.m.	285 c.c.	1·018	Acid	Dark amber	No deposit.	No bands	No precipitate on acidifying and boiling.
,, 11.0 ,,	257 c.c.	1·015	Alkaline	Porter coloured	Slight deposit consisting of granular casts and crystals of triple phosphates.	Methaemoglobin bands stronger than the oxyhaemoglobin bands.	0·82 % (corresponding to 2 gr. of haemoglobin. Chocolate precipitate on acidifying and boiling equals $\frac{1}{5}$ column.
,, 4.0 p.m	198 c.c.	1·016	,,	Amber	No deposit.	No bands	No precipitate on acidifying and boiling.
,, 6.30 ,,	198 c.c.	1·012	,,	,,	,,	..	..
,, 9.0 ,,	198 c.c.	—	,,	,,	,,	..	..
							Total haemoglobin passed during the day equalled 2·6 gr.
8th day, 5.30 a.m	370 c.c.	1·022	Acid	Porter coloured	Brownish deposit about $\frac{1}{10}$th column, consisting of granular casts.	Well marked bands of oxyhaemoglobin and methaemoglobin.	0·8 % (corresponding to 1·9 gr. of haemoglobin). Chocolate precipitate on acidifying and boiling equalling $\frac{1}{5}$ column.
,, 7.30 ,,	114 c.c.	1·018	,,	,,	,, ,,	..	0·8 % (corresponding to 0·9 gr. of haemoglobin .
,, 9.0 ,,	171 c.c.	1·010	,,	Light reddish brown	,, ,,	..	Brownish precipitate on acidifying and boiling equalled $\frac{1}{10}$th column.
,, 11.30 ,,	198 c.c.	1·013	Alkaline	Amber	Very slight deposit.	No bands	Slight turbidity on acidifying and boiling.
,, 1.0 p.m	170 c.c.	1·016	Acid	,,	..	..	..
,, 3.30 ,,	86 c.c.	1·015	,,	Brownish red	,,	..	·3 %
,, 6.0 ,,	142 c.c.	1·013	..	Amber	..	..	Slight turbidity on acidifying and boiling
,, 10.0 ,,	198 c.c.	1·015	..	,,	..	..	..
							Total haemoglobin passed during day equalled 2·8 gr.
9th day, 3.0 a.m.	198 c.c.	1·018	Alkaline	,,	..	..	Slight turbidity on acidifying and boiling.
,, 5.45 ,,	114 c.c.	1·018	,,	,,	..	..	..
,, 7.45 ,,	142 c.c.	1·018	,,	..	..	..	..

Date and time	Amount	Sp. gr.	Reaction	Colour	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	Acid	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	No deposit	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
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[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

Date and time	Amount	Sp. gr.	Reaction	Colour	Microscopical examination of deposit	Spectroscopic examination	Amount of haemoglobin
[illegible] day, 1.20 p.m.	[illegible]	[illegible]	Alkaline	Light red	Slight deposit consisting of [illegible]	Strong [illegible] haemoglobin and methaemoglobin bands	[illegible] corresponding to ·6 gr. of haemoglobin. Chocolate precipitate on acidifying and boiling ¼ column.
„ 3.0 „	[illegible] c.c.	[illegible]	„	Amber	No deposit	No bands	No precipitate on acidifying and boiling.
„ 6.0 „	[illegible] c.c.	[illegible]	„	„	„	„	„ Total haemoglobin passed during the day ·4 gr.
11th day, [illegible] a.m.	[illegible]	1·011	Acid	„	„	„	No precipitate on acidifying and boiling.
„ 11.30 „	200 c.c.	1·012	„	„	„	„	„
„ 2.15 p.m.	180 c.c.	1·012	„	„	„	„	„
„ 4.45 „	120 c.c.	1·01[illegible]	„	„	„	„	„
„ 7.0 „	160 c.c.	1·011	„	„	„	„	„
„ 11.0 „	200 c.c.	1·013	„	„	„	„	„
12th day, 5.0 a.m.	150 c.c.	1·013	„	Dark amber	„	„	„
„ 6.30 „	170 c.c.	1·011	Alkaline	Port wine red	Deposit [illegible], consisting of [illegible] and phosphates	Bands of oxyhaemoglobin distinct	·8 % corresponding to 1·9 gr. of haemoglobin. Chocolate precipitate on boiling ¼ column.
„ 10.30 „	150 c.c.	1·011	Acid	Deep amber	Slight deposit as above	No bands	Very slight precipitate on acidifying and boiling.
„ 11.50 „	200 c.c.	1·012	„	Brownish red		Bands of oxyhaemoglobin present but no methaemoglobin bands	·6 % corresponding to 1·2 gr of haemoglobin. Chocolate precipitate on acidifying and boiling ¼ column
„ 2.0 p.m.	190 c.c.	1·010	„	Dark amber	No deposit	No bands	Very faint cloud on acidifying and boiling.
„ 5.30 „	240 c.c.	1·010	Alkaline	„	„	„	„
„ 8.0 „	115 c.c.	1·010	Acid		„	„	No precipitate on acidifying and boiling.
„ 10.0 „	205 c.c.	1·012	„	„	„	„	„
„ 11.0 „	195 c.c.	1·012	„	„	„	„	„ Total haemoglobin passed during day 1·6 gr.

Date and time.	Amount.	Sp. gr.	Reaction.	Colour.	Appearance and amount of deposit.	Spectroscopic examination.	Amount of haemoglobin.
13th day, 2.0 a.m.	150 c.c.	1·014	Alkaline	Dark amber	No deposit.	No bands.	No precipitate on acidifying and boiling.
,, 4.0 ,,	120 c.c.	1·015	Acid	,,	,,	,,	,,
,, 8.0 ,,	150 c.c.	1·014	Alkaline	Brownish red	Slight deposit of granular casts.	Bands of oxy-haemoglobin just visible.	Slight precipitate on acidifying and boiling.
,, 10.0 ,,	70 c.c.	1·015	,,	,,	,,	Distinct bands of oxyhaemoglobin.	0·6 % (corresponding to 0·4 gr. of haemoglobin). Chocolate precipitate on acidifying and boiling $\frac{1}{6}$ column.
,, 2.0 p.m.	160 c.c.	1·014	Acid	Brownish red	,,	,,	0·6 % (corresponding to 1 gr. of haemoglobin). Chocolate precipitate on acidifying and boiling $\frac{1}{6}$ column.
,, 4.30 ,,	140 c.c.	1·012	,,	,,	,,	,,	0·4 % (corresponding to 0·5 gr. of haemoglobin). Chocolate precipitate on acidifying and boiling $\frac{1}{5}$ column.
,, 7.0 ,,	240 c.c.	1·012	,,	Light brown	,,	No bands.	Faint cloud on acidifying and boiling.
,, 9.30 ,,	140 c.c.	1·014	,,	,,	No deposit.	,,	,, Total haemoglobin passed during day 1·9 gr.
14th day, 1.0 a.m.	160 c.c.	1·014	Alkaline	,,	,,	,,	Faint cloud on acidifying and boiling.
,, 6.0 ,,	180 c.c.	1·015	,,	,,	,,	,,	,,
,, noon	240 c.c.	1·015	Acid	,,	,,	,,	,,
,, 5.30 p.m.	240 c.c.	1·015	,,	,,	,,	,,	,,
,, 7.0 ,,	210 c.c.	1·015	,,	Amber	,,	,,	No precipitate on acidifying and boiling.
,, 10.0 ,,	420 c.c.	1·008	Alkaline	,,	,,	,,	,,
15th day	1310 c.c.	1·015	Acid	,,	,,	,,	,,
16th day	1210 c.c.	1·014	,,	,,	,,	,,	,,
17th day	1413 c.c.	1·011	,,	,,	,,	,,	,,
18th day	1320 c.c.	1·011	,,	,,	,,	,,	,,
19th day	1230 c.c.	1·012	,,	,,	,,	,,	,,

BLACKWATER FEVER. CASE 11.

Male, thirty-eight years of age. Locomotive inspector. European.

First came to Nyasaland nine months ago, and has lived chiefly in Port Herald and the Lower Shiré district. Previous to coming to Nyasaland he had spent five years in Rhodesia. During the last nine months he has had a good deal of malaria but has never previously had blackwater fever. He has very rarely taken any quinine. During the last week he has been very unwell, and two days ago he took ten grains of quinine. The next day he felt no better, but took no more quinine.

1st day of present illness. At 9 a.m. he felt very ill and took ten grains of quinine. At 10-15 a.m. he passed porter-coloured urine and again at 11-30 a.m. T. 103° F. During the remainder of the day patient was very restless vomited several times, and passed no more urine.

2nd day. Slept a little during the night and took large quantities of fluid by the mouth. At 10 a.m. he passed a little urine, which was porter coloured. Vomited several times. Skin and conjunctivae of a very distinct lemon yellow tint. Spleen easily palpable. T. 105° F. At 5 p.m. T. 101° F.

3rd day. Had a very bad night; passed no urine during the night. Continued to take large quantities of fluid by the mouth. At 3-30 p.m. he passed 10 c.cm. of dark red urine. At 6 p.m. the skin was very sallow and the conjunctivae lemon-yellow. T. 101° F. Vomiting and hiccough very troublesome.

4th day. Slept very little. T. 100° F. Urine amber coloured. The total amount of urine passed during the day was 48 c.cm.

5th day. Condition of patient was unchanged. Urine amber coloured. Temperature normal. The total amount of urine passed during the day was 92 c.cm.

6th day. Total amount of urine passed to-day was 73 c.cm.

7th day. Vomiting and hiccough continue. Total urine passed during day was 93 c.cm.

8th day. Patient felt better, slept well, pulse and temperature normal, and the vomiting was not so troublesome. Amount of urine passed during day was 85 c.cm.

9th day. Patient had a good night and slept well. At 7 a.m. about 8 c.cm. of urine were passed. After this no more urine was voided, and very severe dyspnoea set in about 4 p.m. Finally at 7 p.m. patient died during an attack of dyspnoea.

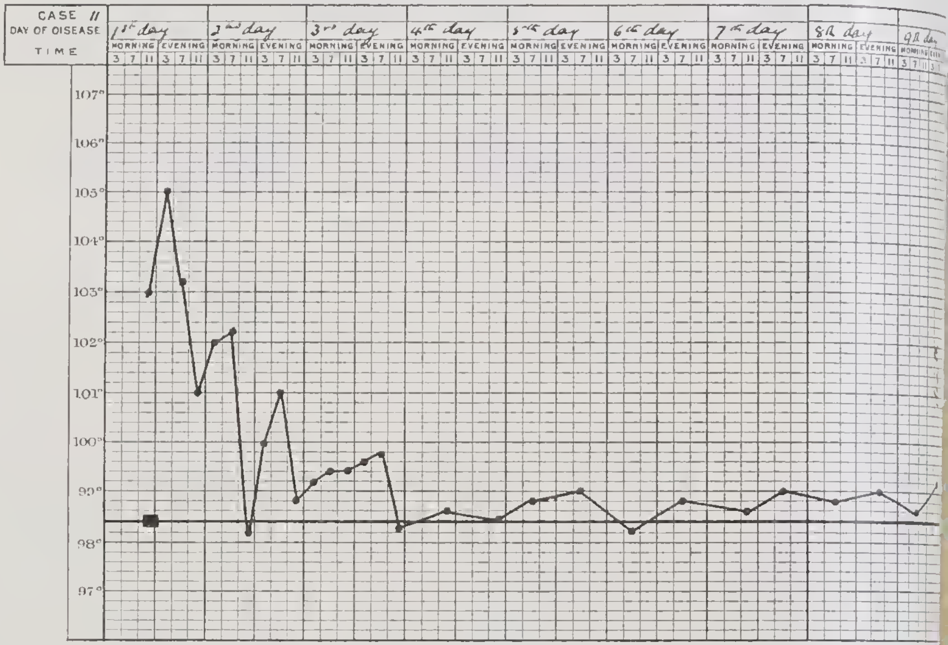

FIG. 76. Blackwater Fever. Case 11. Temperature Chart.

Condition of blood. 3rd day. At 7 p.m. patient's oxalated plasma was of a deep orange colour with no reddish tint, and gave marked bands of oxyhaemoglobin in a column 18 mm. high, representing 0·25 per cent. of dissolved haemoglobin. Methaemoglobin bands were

No. of experiment.	COMPOSITION OF MIXTURE OF RED BLOOD CELLS AND QUININE SOLUTION.			Haemolysis.
	2·5 % emulsion of red blood cells.	0·9 % solution of sodium chloride.	1·92 % solution of quinine bihydrochloride.	
1	0·5 c.c.	2·4 c.c.	0·1 c.c.	Complete at end of 1 hour
2	0·65 c.c.	3·0 c.c.	0·1 c.c.	Complete at end of 2 hours
3	0·8 c.c.	3·6 c.c.	0·1 c.c.	Complete at end of 2½ ho
4	1·05 c.c.	4·6 c.c.	0·1 c.c.	Trace at end of 3 hours

also distinct. No autolysin was present in the plasma and no malarial parasites or pigmented leucocytes were found in the blood. On examination with the aid of the haemocrit the blood was found to contain 16·3 per cent. by volume of red cells. The above observations were made at 37° C. with the patient's red blood cells suspended in 0·9 per cent. sodium chloride solution, to which quinine bihydrochloride had been added.

4th day. At 10 a.m. patient's oxalated plasma was of a deep orange colour, but not so deep as yesterday. It gave faint oxyhaemoglobin bands, 0·16 per cent. of haemoglobin being present in solution. No autolysin was present in the plasma, and no malarial parasites or pigmented leucocytes were found in the blood.

7th day. At 10-30 a.m. patient's oxalated plasma was of a light orange colour. Oxyhaemoglobin bands were faint, the plasma containing 0·1 per cent. of haemoglobin in solution. No autolysin was present in the plasma, and no parasites or pigmented leucocytes were found in the blood. On examining with the aid of the haemocrit the blood was found to contain 14·8 per cent. by volume of red cells. The following observations were made at 37° C., with the patient's red blood cells suspended in 0·9 per cent. sodium chloride solution, to which quinine bihydrochloride had been added :

No. of experiment.	COMPOSITION OF MIXTURE OF RED BLOOD CELLS AND QUININE SOLUTION.			Haemolysis.
	2·5 % emulsion of red blood cells.	0·9 % solution of sodium chloride.	1·92 % solution of quinine bihydrochloride.	
1	0·4 c.c.	2·0 c.c.	0·1 c.c.	Complete at end of 1 hour
2	0·5 c.c.	2·4 c.c.	0·1 c.c.	Complete at end of 1 hour
3	0·65 c.c.	3·0 c.c.	0·1 c.c.	Complete at end of 1½ hours
4	0·8 c.c.	3·6 c.c.	0·1 c.c.	Complete at end of 2 hours
5	1·05 c.c.	4·6 c.c.	0·1 c.c.	Trace at end of 3 hours
6	1·3 c.c.	5·6 c.c.	0·1 c.c.	Nil at end of 3 hours
7	1·5 c.c.	6·4.c.c.	0·1 c.c.	Nil at end of 3 hours
8	1·3 c.c.	4·6 + 1 c.c. plasma	0·1 c.c.	Nil at end of 3 hours

Condition of the spleen.— 6th day. At 11 a.m. a spleen puncture was made. Phagocytosis of red cells (by less than 0·1 per cent. of the white cells) was observed. No malarial parasites or pigment found.

Condition of urine.

Date and time.	Amount.	Sp. gr.	Reaction.	Colour.	Appearance and amount of deposit.	Spectroscopic examination.	Amount of haemoglobin.
1st day, 10.15 a.m.	225 c.c.	1·017	—	Porter coloured	Large deposit consisting of granular casts and débris and a few renal epithelial cells.	Very strong oxyhaemoglobin bands. No methaemoglobin bands.	2 % (corresponding to 4·5 gr. of haemoglobin). Chocolate precipitate on acidifying and boiling 2·6 % haemoglobin.
,, 11.30 ,,	140 c.c.	1·017	—	,,	,, ,,	,,	2 % (corresponding to 2·8 gr. of haemoglobin). Chocolate precipitate on acidifying and boiling equalled 2·6 % haemoglobin.
2nd day, 10.0 a.m.	10 c.c.	1·017	—	,,	,, ,,	,,	2 % (corresponding to 0·2 gr. of haemoglobin).
							Total haemoglobin passed during two days was 7·5 gr.
3rd day, 3.30 p.m.	10 c.c.	—	Alkaline	,,	,, ,,	Both oxyhaemoglobin and methaemoglobin bands present.	1·5 % (corresponding to 0·15 gr. of haemoglobin). Chocolate precipitate on acidifying and boiling was 2·1 % of haemoglobin.
,, 7.0 ,,	12 c.c.	—	,,	Brownish red	,, ,,	,,	0·5 % (corresponding to 0·05 gr. of haemoglobin). Chocolate precipitate on acidifying and boiling equalled 1 % haemoglobin. Total haemoglobin passed during day was 0·2 gr.
4th day, 3.45 a.m.	8 c.c.	—	,,	,,	Deposit on centrifugalising slight, consisting of few granular casts and epithelial cells. Few red cells (about one in a field).	Very faint bands of both oxyhaemoglobin and methaemoglobin.	Light brown precipitate on acidifying and boiling; $\frac{3}{5}$ column.

Date and time.	Amount.	Sp. gr.	Reaction.	Colour.	Appearance and amount of deposit.	Spectroscopic examination.	Amount of haemoglobin.
4th day, 7.0 a.m.	5 c.c.	—	Alkaline	Amber	Deposit as above but red cells rather more numerous.	No bands	Whitish precipitate, on acidifying and boiling, $\frac{1}{4}$ column.
,, 11.0 ,,	10 c.c.	—	,,	,,	,, ,,	,,	,,
,, 4.0 p.m.	11 c.c.	—	,,	,,	,, ,,	,,	,,
,, 6.30 ,,	4 c.c.	—	,,	,,	,, ,,	,,	,,
,, 10.0 ,,	10 c.c.	—	,,	,,	,, ,,	,,	,,
5th day, 6.30 a.m.	30 c.c.	1·008	,,	,,	Slight deposit on centrifugalising consisting of granular casts, epithelial cells, red cells, and leucocytes.	,,	Whitish precipitate, on acidifying and boiling, $\frac{1}{5}$ column.
,, 10.0 ,,	10 c.c.	—	,,	,,	,, ,,	,,	,,
,, 2.30 p.m.	14 c.c.	1·008	,,	,,	,, ,,	,,	,,
,, 5.30 ,,	10 c.c.	—	,,	,,	,, ,,	,,	,,
,, 7.0 ,,	8 c.c.	—	,,	,,	,, ,,	,,	,,
,, 10.30 ,,	20 c.c.	1·008	,,	,,	,, ,,	,,	,,
6th day, 3.0 a.m.	12 c.c.	—	,,	,,	Deposit on centrifugalising slight, consisting of granular casts and renal epithelial cells and a few red cells and crystals of triple phosphates.	,,	Whitish precipitate on acidifying and boiling equalled $\frac{2}{5}$ column.
,, 9.45 ,,	18 c.c.	—	,,	,,	,, ,,	,,	,,
,, 7.0 p.m.	15 c.c.	—	,,	,,	,, ,,	,,	,,
,, 11.25 ,,	16 c.c.	—	,,	,,	,, ,,	,,	,,
,, 11.40 ,,	12 c.c.	—	,,	,,	,, ,,	,,	,,
7th day, 3.40 a.m.	18 c.c.	—	,,	,,	,, ,,	,,	,,
,, 7.35 ,,	18 c.c.	—	,,	,,	,, ,,	,,	,,
,, 9.0 ,,	8 c.c.	—	,,	,,	,, ,,	,,	,,
,, 10.0 ,,	5 c.c.	—	,,	,,	,, ,,	,,	,,
,, 4.0 p.m.	13 c.c.	—	,,	,,	,, ,,	,,	,,
,, 6.30 ,,	6 c.c.	—	,,	,,	,, ,,	,,	,,
,, 11.15 ,,	24 c.c.	—	,,	,,	,, ,,	,,	,,
8th day, 6.10 a.m.	13 c.c.	—	,,	,,	,, ,,	,,	,,
,, 7.20 ,,	9 c.c.	—	,,	,,	,, ,,	,,	,,
,, 10.0 a.m.	10 c.c.	—	,,	,,	,, ,,	,,	,,
,, 1.5 p.m.	23 c.c.	—	,,	,,	,, ,,	,,	,,
,, 4.50 ,,	20 c.c.	—	,,	,,	,, ,,	,,	,,
,, 6.10 ,,	9 c.c.	—	,,	,,	,, ,,	,,	,,
9th day, 7.0 a.m.	8 c.c.	—	,,	,,	,, ,,	,,	,,

Post-mortem examination. Body well nourished, no distinct yellowness of the skin. No oedema of the subcutaneous tissues. About 600 c.cm. of fluid in the peritoneal cavity. The spleen was considerably enlarged. The liver was enlarged and pale. The kidneys were considerably enlarged and congested; capsules slightly adherent; cortex swollen but otherwise not markedly altered; pyramids very dark, exhibiting a striated appearance; the pelvis of the kidney normal in aspect. The heart was slightly dilated; no excess of fluid in the pericardium; heart substance natural in aspect; valves of heart healthy. Pleural cavities free from fluid. Lungs not congested or oedematous. Mucous membrane of stomach and oesophagus injected. The suprarenals and pancreas presented no change. Bladder healthy in aspect; it contained about 10 c.cm. of amber coloured urine.

Microscopical examination.—The kidneys contained numerous brownish plugs or casts within the uriniferous and collecting tubules. These plugs were made up of coarse granules 5μ to 3μ in diameter, arranged singly or in groups, partially filling the lumen of the tubules and frequently accompanied by free epithelial cells and finely granular or flocculent deposit. Further details are given on pp. 107-119. The renal tubules were dilated. The interstitial tissue presented no change in aspect. The blood vessels contained no malarial pigment. The liver showed slight interlobular cirrhosis, but no fatty change and no malarial pigment. No malarial parasites or pigment were found in the spleen.

BLACKWATER FEVER. CASE 12.

Male, thirty-four years of age. Fitter. Indian.

Has been in Nyasaland for the past eleven months, having previously lived some time in Uganda. During the last six months he has suffered from fever two or three times every month. He has never previously had blackwater fever. Does not habitually take quinine, but occasionally when he has fever he takes five or ten grains. He commenced to be ill yesterday, and took five grains of quinine.

1st day. Felt no better this morning. At noon he had a rigor, and took five grains of quinine. At 4-45 p.m. he passed blackwater.

2nd day. Patient felt rather better to-day. T. 99·6° F. Spleen slightly enlarged and just palpable below costal margin. No vomiting.

3rd day. Patient appeared well to-day. The temperature was normal, and the urine amber coloured. Quinine sulphate in solution was given in three doses of one grain each.

4th day. Quinine was given as above in three doses of two grains each.

5th day. Three doses of three grains of quinine were administered to-day. The patient continued well; there was no relapse of the blackwater.

6th day. Three doses of four grains of quinine were given to-day.

7th day. From to-day the patient took five grains of quinine three times daily. The patient's recovery was uninterrupted.

Condition of blood.—2nd day. At 4-30 p.m. patient's oxalated plasma was of a dark orange colour. It gave distinct oxyhaemoglobin bands, 0·2 per cent. of haemoglobin being present in solution. On examining with the aid of the haemocrit, the blood was found to contain 33 per cent. by volume of red cells. On testing for haemolysin complete haemolysis occurred, but the plasma, which had been kept for twenty-four hours, was found to contain a considerable number of bacteria. No malarial parasites or pigmented leucocytes were found in the blood.

3rd day. At 10-30 a.m. patient's oxalated plasma was of an amber colour. It gave faint oxyhaemoglobin bands, when examined in a column eighteen millimetres high, 0·1 per cent. of haemoglobin being present in solution. No autolysin was present, and no malarial parasites or pigmented leucocytes were found in the blood.

Condition of the spleen. 2nd day. At 4-30 p.m. a spleen puncture was made. Phagocytosis of red cells (by about 3 per cent. of white cells) was observed. No malarial parasites or pigment found.

3rd day. At 10-30 a.m. a spleen puncture was made. Phagocytosis of red cells (by about 3 per cent. of white cells) was observed. No malarial parasites or pigment found.

Condition of urine.

Date and time.	Amount.	Sp. gr.	Reaction.	Colour.	Appearance and amount of deposit.	Spectroscopic examination.	Amount of haemoglobin.
1st day, 11.0 p.m.	—	1·015	Alkaline	Very dark red	Considerable deposit on centrifugalising consisting of granular casts, epithelial cells and crystals. No red cells.	Strong bands of oxyhaemoglobin and methaemoglobin.	2·2 % chocolate precipitate, on acidifying and boiling, $\frac{3}{4}$ column.
2nd day, 1.0 a.m.	—	1·008	,,	,,	,, ,,	,,	,,
,, 7.0 ,,	200 c.c.	1·009	Acid	Claret coloured	,, ,,	,,	0·34 % chocolate precipitate, on acidifying and boiling, $\frac{1}{5}$ column.
,, 2.0 p.m.	—	1·012	,,	Light claret coloured	,, ,,		0·2 %.
,, 4.0 ,,	—	1·006	,,	Brownish	,, ,,	Oxyhaemoglobin but no methaemoglobin bands visible.	0·1 % chocolate precipitate, on boiling, $\frac{1}{12}$ column.
,, 5.0 ,,	—	1·012	Alkaline	Light brown	Slight deposit consisting of granular and hyaline casts and renal epithelial cells.	,,	0·8 % chocolate precipitate, on acidifying and boiling, $\frac{1}{15}$ column.
,, 6.45 ,,	—	1·015	Acid	,,	,, ,,	,,	Slight precipitate on acidifying and boiling.
,, 8.0 ,,	170 c.c.	1·014	,,	Amber	Very slight deposit.	No bands.	,,
3rd day	—	—	—	,,	No deposit.	,,	No precipitate on acidifying and boiling.

Subsequent specimens of urine were amber coloured and normal.

BLACKWATER FEVER. CASE 13.

Male, twenty-nine years of age. Missionary. European.

Has been in Nyasaland for five years. From time to time had attacks of malaria, which were not treated with quinine. Had a slight attack of blackwater above a year ago.

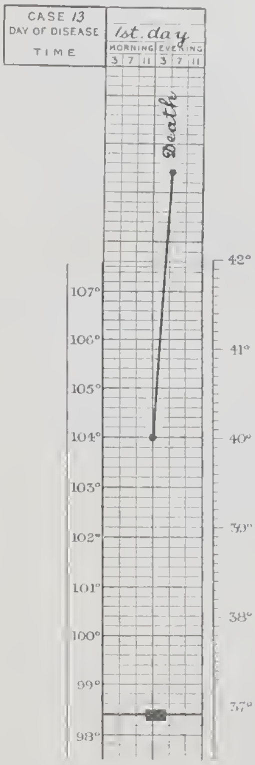

FIG 77. Blackwater Fever, Case 13. Temperature Chart.

Took four grains of quinine yesterday. Spent the day working, in a hot sun, at brick-making. Stated to have been quite well.

This morning took four grains of quinine. Remained well till mid-day. Became seriously ill at 1 p.m., when he vomited black material like coffee grounds. Temperature was then 40° C. (104° F.) He was then much distressed, and complained of pain in the abdomen, putting his hands to the hypogastric region. Passed red urine, described as 'looking like blood.' He became delirious and violent, and four men were required to restrain him. In his delirium bit the forearm of another missionary. Temperature continued to rise to

43° C. (109·4° F.). Patient's face and hands became deeply yellow. Died at 5 p.m. Not seen by myself till 11 p.m., when the urine and vomit had been thrown away. Face and hands were of a markedly brownish yellow colour. Post mortem examination not permitted.

BLACKWATER FEVER. CASE 14.

Male, thirty-nine years of age. Store assistant. Indian.

Has been in Nyasaland for five years. He has had a great deal of fever, his temperature frequently reaching 102° or 103° F. About fifteen months ago he had his first attack of blackwater fever. Has not taken quinine regularly, but only occasionally when ill.

1st day of illness. Has been feeling unwell for some time, but only became seriously ill to-day, when his temperature rose to 100° F. He had a severe headache, took ten grains of quinine sulphate, and went to bed.

2nd day. Condition unchanged. T. 99·8° F. Took five grains of quinine. In the evening his temperature had risen to 101° F., and he had another five grains of quinine. At 10 p.m. he had a severe rigor. T. 105·4° F. Shortly afterwards he passed porter coloured urine. Vomiting was very troublesome.

3rd day. Patient had another severe rigor this morning. T. 106° F. Severe vomiting all day.

4th day. Condition unchanged this morning. Skin and conjunctivae of a lemon yellow colour, spleen just palpable. T. 103° F. The vomiting was still severe, but the urine was evidently clearing in the evening.

5th day. Patient much better, urine clear, vomiting less troublesome, temperature normal.

Subsequently the patient's recovery was uninterrupted.

Condition of blood. 4th day. At 5-30 p.m. patient's oxalated plasma was of a reddish colour; it gave in a column eighteen millimetres high distinct bands of oxyhaemoglobin and methaemoglobin, equalling 0·57 per cent. of haemoglobin in solution. No malarial parasites or pigmented leucocytes were found in the blood.

5th day. At 10-45 a.m. patient's oxalated plasma was of a deep orange colour with no red tint, containing 0·18 per cent. of haemoglobin. Methaemoglobin bands were also distinct. No autolysin present in the plasma, and no malarial parasites or pigmented leucocytes were found in the blood. With the aid of the haemocrit the patient's blood was found to contain 33·3 per cent., by volume, of red cells. The following observations were made at 37° C. with the patient's red blood cells suspended in 0·9 per cent. sodium chloride solution, to which quinine bihydrochloride had been added:

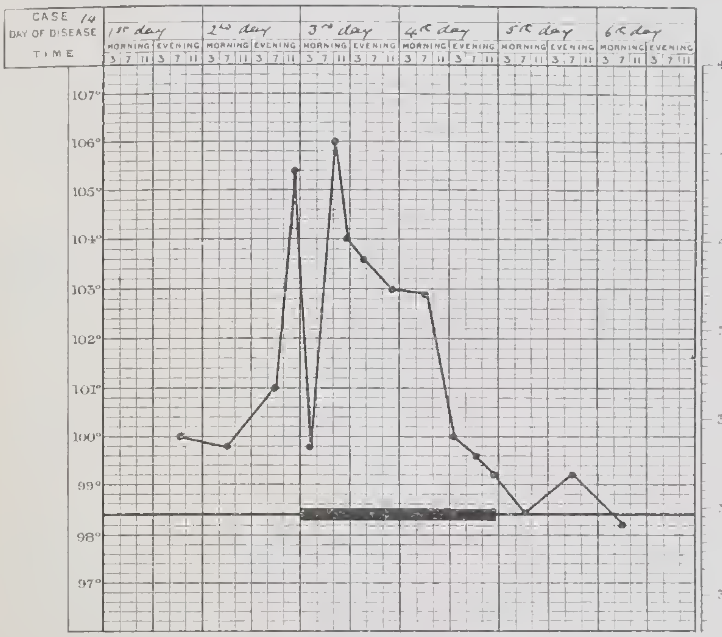

FIG. 78. Blackwater Fever, Case 14. Temperature Chart.

No. of experiment.	Composition of Mixture of Red Blood Cells and Quinine Solution.			Haemolysis.
	2·5 % emulsion of red blood cells	0·9 % solution of sodium chloride	1·92 % solution of quinine bihydrochloride	
1	0·5 c.c.	2·4 c.c.	0·1 c.c.	Complete at end of 1 hour
2	0·65 c.c	3·0 c.c.	0·1 c.c.	Complete at end of 2 hours
3	0·8 c.c.	3·6 c.c.	0·1 c.c.	Complete at end of 2 hours
4	1·05 c.c.	4·6 c.c.	0·1 c.c.	Trace at end of 3 hours
5	1·3 c.c.	5·6 c.c.	0·1 c.c.	Nil at end of 3 hours

Condition of spleen.—4th day. At 5-30 p.m. a spleen puncture was made. Phagocytosis of red cells (by less than 1 per cent. of white cells) was observed. No malarial parasites or pigment found.

Condition of urine

Date and time	Volume	Sp. gr.	Proteins	Colour	Appearance and microscopic features	Spectroscopic examination	Amount of haemoglobin
1st day, [illegible]	[illegible]	1·0[illegible]	Nil	[illegible]	Deposit of [illegible] granular casts. No red cells		Chocolate precipitate on acidifying and boiling less than [illegible]% of haemoglobin (corresponding to [illegible] g of haemoglobin)
[illegible]	[illegible]	[illegible]	—	Dark red			Chocolate precipitate on acidifying and boiling less than [illegible]% of haemoglobin (corresponding to [illegible] g of haemoglobin)
3rd day, [illegible]	[illegible]	[illegible]	[illegible]	[illegible]			Chocolate precipitate on acidifying and boiling less than [illegible]% of haemoglobin (corresponding to [illegible] g of haemoglobin) Total "haemoglobin" passed during [illegible] was 47·5 g
4th day, [illegible]	[illegible]	[illegible]		Clear	—	—	Chocolate precipitate on acidifying and boiling less than [illegible]% of haemoglobin (corresponding to [illegible] g of haemoglobin)
[illegible]	[illegible]	[illegible]			[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]		Brownish red	—	—	[illegible]
[illegible]	[illegible]	[illegible]		[illegible]	—	[illegible]	[illegible]

BLACKWATER FEVER. CASE 14a.

Male, thirty-nine years of age. Store assistant. Indian.

See history of preceding attack (Case 14). Has remained in fairly good health since his last attack of blackwater fever three months ago.

1st day. He felt unwell this morning, and took ten grains of quinine. At 2-30 p.m. he was seized with a feeling of uneasiness, accompanied by vomiting and shivering. At 3 p.m. he passed red urine. T. 101·4° F.

2nd day. Patient remained ill all day, passing porter coloured urine; vomiting very severe.

3rd day. Shortly after midnight he had a rather severe rigor. T. 102° F. The vomiting continued all morning, but ceased in the afternoon, when the patient felt much better. The urine, which had been amber coloured in the morning, cleared up later in the day, and towards evening was amber in colour.

4th day. Patient much better; the temperature was normal, and the urine dark amber coloured.

Subsequently the patient's recovery was uninterrupted.

Condition of blood.—3rd day. At 12-30 p.m. blood was withdrawn from the finger. The blood appeared watery, and patient's oxalated plasma was of a deep orange colour with no red tint, containing 0·2 per cent. of haemoglobin in solution. Very faint bands of methaemoglobin could be detected in a column eighteen millimetres high. On examination with the aid of the haemocrit, the patient's blood was found to contain 24 per cent. by volume of red cells. A haemoglobinometer reading of 33 divisions of von Fleischl's scale was obtained (equivalent to 0·1 per cent. of wet red cells)

$$\frac{\text{Haemoglobin}}{\text{Volume}} = 1{\cdot}37.$$

Condition of urine.

Date and time.	Amount.	Sp. gr.	Reaction.	Colour.	Appearance and amount of deposit.	Spectroscopic examination.	Amount of haemoglobin.
1st day, 3.0 p.m.	280 c.c.	—	Alkaline	Porter coloured	Deposit on centrifugalising about $\frac{1}{50}$ column of brownish white colour, consisting of granular casts, the granules being of varying degrees of coarseness and brown in colour, though not deeply tinted. Nuclei are seen in some of the casts. The longest casts measure 252 μ × 54 μ. No red cells, stromata or leucocytes.	Oxyhaemoglobin bands but no methaemoglobin bands present.	1·8 % (corresponding to 8·6 gr. of haemoglobin). Chocolate coloured precipitate on acidifying and boiling was less than 2·5 % of haemoglobin. Total haemoglobin passed during the day was 8·6 gr.
,, 8.30 ,,	200 c.c.						
2nd day, 3.45 a.m.	770 c.c.	—	Alkaline	Porter coloured	,, ,,	,,	1·2 % (corresponding to 9·3 gr. of haemoglobin). Chocolate precipitate on acidifying and boiling less than 1·3 % of haemoglobin. Total haemoglobin passed during the day was 9·3 gr.
,, 8.30 ,,							
,, 10.15 ,,							
, 2.20 p.m.							
,, 5.30 ,,							
,, 7.45 ,,							
,, 12.30 a.m.							
3rd day, 1.45 a.m.	150 c.c.	—	,,	,,	,, ,,	,,	0·5 % (corresponding to 2·3 gr. of haemoglobin).
,, 5.30 ,,	140 c.c.						
,, 10.15 ,,	150 c.c.						
,, 12.30 p.m.	50 c.c.	—	Neutral	,,	,, ,,	,,	0·25 % (corresponding to 0·1 gr. of haemoglobin).
,, 6.0 ,,	120 c.c.	—	Alkaline	Brownish amber coloured	Very little deposit on centrifugalising.	No bands.	Dark brown precipitate on acidifying and boiling less than 0·4 % of haemoglobin. Total haemoglobin passed during day 2·4 gr.
4th day, 6.0 a.m.	610 c.c.	—	—	Dark amber coloured	No deposit.	,,	No precipitate on acidifying and boiling.
,, 11.45 ,,	360 c.c.	—	—	Amber coloured	,,	,,	,,

Subsequent specimens of the urine were amber coloured and normal.

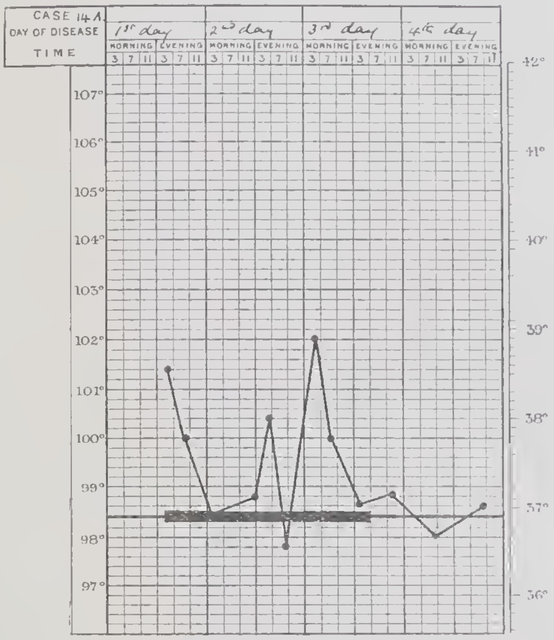

FIG. 79. Blackwater Fever, Case 14a. Temperature Chart.

BLACKWATER FEVER. CASE 15.

Male, twenty-five years of age. Store-keeper. European.

Has lived in Nyasaland for six months and has never been in the tropics previously. During this time he has had a good deal of fever. Stated that he had taken thirty grains of quinine a week regularly since he has been in Nyasaland, and that in spite of this he has suffered considerably from malaria. He has never used a mosquito net. Began to be ill three or four days ago. Felt feverish, had anorexia and was unable to sleep. Three days before the attack commenced he took ten grains of quinine. The next day he took five grains of quinine, and the day before the attack he took five grains of quinine.

1st day of attack. Got up about 7 a.m., and took ten grains of quinine. At 4 p.m. he noticed that his urine was porter coloured.

This was the first intimation he had that he was suffering from blackwater fever. He had no previous rigor. T. 103·6° F.

2nd day. Patient had a bad night, vomiting very troublesome, skin of a marked yellowish brown colour. T. 103° F. Twenty-three ounces of physiological saline solution were injected subcutaneously.

3rd day. General condition improved; the vomiting was not so troublesome, and the temperature had fallen to 100° F. Twenty-three ounces of physiological saline solution were again injected subcutaneously.

4th day. Patient was much better, and his recovery continued uninterruptedly.

Condition of blood. 1st day. At 9-30 p.m. patient's oxalated plasma was of a dark orange colour with a faint red tint, containing 0·56 per cent. of haemoglobin. No autolysin was present in the plasma, and no parasites or pigmented leucocytes were found in the blood. The blood had a watery appearance. The following observations were made at 37° C. with the patient's red blood cells suspended in 0·9 per cent. sodium chloride solution to which quinine bihydrochloride had been added:—

No. of experiment.	COMPOSITION OF MIXTURE OF RED BLOOD CELLS AND QUININE SOLUTION.			Haemolysis.
	2·5 % emulsion of red blood cells	0·9 % solution of sodium chloride	1·92 % solution of quinine bihydrochloride	
1	0·5 c.c.	2·4 c.c.	0·1 c.c.	Complete at end of 1 hour
2	0·65 c.c.	3·0 c.c.	0·1 c.c.	Complete at end of 2 hours
3	0·8 c.c.	3·6 c.c.	0·1 c.c.	Complete at end of 2½ hours
4	1·05 c.c.	4·6 c.c.	0·1 c.c.	Trace at end of 3 hours
5	1·3 c.c.	5·6 c.c.	0·1 c.c.	Nil at end of 3 hours

2nd day. At 1 p.m. patient's oxalated plasma was of a dark orange colour with no red tint, containing 0·42 per cent. of haemoglobin. No autolysin was present in the plasma.

Condition of the spleen.—1st day. At 10 p.m. a spleen puncture was made. Phagocytosis of red cells (by less than 1 per cent. of white cells) was observed. No malarial parasites or pigment found.

Condition of urine.

Date and time.	Amount.	Sp. gr.	Reaction.	Colour.	Appearance and amount of deposit.	Spectroscopic examination.	Amount of haemoglobin.
1st day. 7.0 a.m.	—	—	—	Amber	—	—	—
,, noon	—	—	—	Red	—	—	—
,, 4.0 p.m.	—	—	—	,,	—	—	—
,, 6.0 ,,	70 c.c.	1·026	Acid	Porter coloured	Deposit on centrifugalising about $\frac{1}{60}$ column, brown coloured, consisting of granular debris and casts, also a few hyaline and epithelial casts and polygonal and columnar epithelial cells. No squamous epithelial cells and no red cells, leucocytes or bacteria.	Strong oxy-haemoglobin and methaemoglobin bands.	2 % (corresponding to 1·4 gr. of haemoglobin). Chocolate coloured precipitate on acidifying and boiling equalled 3·75 % of haemoglobin.
,, 9.10 ,,	89 c.c.	1·015	,,	,,	,, ,,	Strong oxy-haemoglobin and methaemoglobin bands.	1·3 % (corresponding to 1·2 gr. of haemoglobin). Chocolate precipitate on acidifying and boiling equalled 2·75 gr. of haemoglobin.
							Total haemoglobin passed during the day was 2·6 gr.
2nd day, 3.45 a.m.	360 c.c.	1·020	,,	,,	Deposit on centrifugalising about $\frac{1}{10}$ column, as above.	,,	1·3 % (corresponding to 4·6 gr. of haemoglobin). Chocolate precipitate on acidifying and boiling equalled 3 % of haemoglobin.
,, 7.0 a.m. until 7.10 p.m.	446 c.c.	1·016	,,	,,	,, ,,	,,	0·7 % (corresponding to 3·4 gr. of haemoglobin). Chocolate precipitate on acidifying and boiling equalled 2·5 % of haemoglobin.
							Total haemoglobin passed during day was 8·1 gr.

Date and time	Amount	Sp. gr.	Reaction	Colour	Appearance and amount of deposit	Spectroscopic examination	Amount of haemoglobin passed
3rd day, 5.45 a.m.	359 c.c.	1·018	Neutral	Porter coloured	Deposit on centrifugalising about $\frac{1}{10}$ column, as above.	Strong oxy-haemoglobin and methaemoglobin bands.	0·7 % (corresponding to 2·5 gr. of haemoglobin). Chocolate precipitate on acidifying and boiling was 1·25 % of haemoglobin.
,, 7.0 ,,	450 c.c.	1·016	Alkaline	,,	Deposit on centrifugalising $\frac{1}{5}$ column, consisting of granular debris and casts and a few epithelial casts and cells.	Oxyhaemoglobin and methaemoglobin bands present.	1·1 % (corresponding to 4·5 gr. of haemoglobin). Chocolate coloured precipitate on acidifying and boiling equalled less than 1·6 % of haemoglobin.
,, 10.30 p.m.	470 c.c.	1·015	,,	Light reddish brown	Deposit on centrifugalising $\frac{1}{60}$ column as above.	Oxyhaemoglobin but no methaemoglobin bands present.	0·26 % (corresponding to 1·3 gr. of haemoglobin). Chocolate coloured precipitate on acidifying and boiling equalled less than 0·6 % of haemoglobin. Total haemoglobin passed during the day equalled 8·3 gr.
4th day, 8.15 a.m.	900 c.c.	1·026	,,	Light brown	,, ,,	,,	—
,, 2.20 p.m.	—	—	,,	,,	,, ,,	,,	Whitish precipitate on acidifying and boiling.
,, 5.45 ,,	—	—	Acid	Amber	No deposit	No bands.	Trace of precipitate on acidifying and boiling.
,, 9.15 ,,	—	—	,,	,,	,,	,,	

Subsequent specimens of the urine were amber coloured and normal.

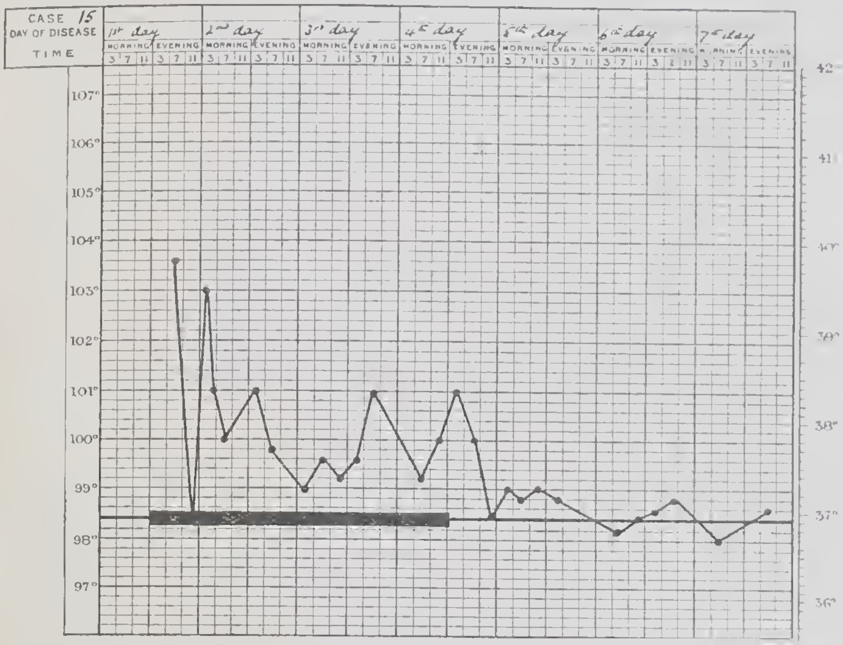

FIG. 80. Blackwater Fever, Case 15. Temperature Chart.

BLACKWATER FEVER. CASE 16.

Male, thirty-five years of age. Cook. Indian.

Has lived in Nyasaland for four years, and during this time has had a good deal of fever, but this has not often been so severe as to necessitate his giving up work. Has never previously had blackwater fever. Has not taken quinine regularly. He began to be ill with fever and headache four days ago. Quinine bihydrochloride was given in solution in doses of five grains three times daily. He continued to take this solution of quinine in similar doses during the next three days.

1st day. Patient passed blackwater this morning. He was obviously very ill, vomiting incessantly. The skin and conjunctivae were yellowish. T. 101·6° F. He had considerable diarrhoea, and during the day he passed six black tarry stools and stated he had been passing similar stools for the past three or four days.

2nd day. His condition was unchanged. The spleen could just be felt below the costal margin.

3rd day. The urine was still red coloured, and the vomiting was very troublesome. After to-day patient was unable to pass his urine, and a catheter had to be used to draw it off. On passing a catheter it was found that he had a urethral stricture, which rendered the passage of the instrument difficult.

4th day. General condition bad, pulse weak and frequent, patient very lethargic. The urine, which had cleared up during the night, was amber coloured, and contained considerable quantities of albumin.

5th day. Condition unchanged.

6th day. The quantity of urine secreted has diminished considerably in amount, being only 500-600 c.cm. The vomiting persists, and the pulse is very frequent and irregular.

10th day. Patient died at 1 a.m. this morning. During the last few days he had sunk into a comatose condition.

Condition of blood.—3rd day. At 10 a.m. patient's oxalated plasma was of a dark orange colour with no red tint, containing 0·2 per cent. of haemoglobin in solution. No methaemoglobin bands were visible in a column of the oxalated plasma eighteen millimetres high. No autolysin was present in the plasma, and no malarial parasites or pigmented leucocytes were found in the blood. The following observations were made at 37° C. with the patient's red blood cells suspended in 0·9 per cent. sodium chloride solution to which quinine bihydrochloride had been added:—

No. of experiment.	COMPOSITION OF MIXTURE OF RED BLOOD CELLS AND QUININE SOLUTION.			Haemolysis.
	2·5 % emulsion of red blood cells.	0·9 % solution of sodium chloride.	1·92 % solution of quinine bihydrochloride.	
1	0·65 c.c.	3·0 c.c.	0·1 c.c.	Complete at end of 2 hours.
2	0·8 c.c.	3·6 c.c.	0·1 c.c.	Complete at end of 2½ hour
3	1·05 c.c.	4·6 c.c.	0·1 c.c.	Partial at end of 3 hours.
4	1·3 c.c.	5·6 c.c.	0·1 c.c.	Trace at end of 3 hours.

Condition of spleen.—3rd day. At 11 a.m. a spleen puncture was made. Phagocytosis of red cells (by about 1 per cent. of the white cells) was observed. No malarial parasites or pigment found.

Condition of urine.

Date and time.	Amount.	Sp. gr.	Reaction.	Colour.	Appearance and amount of deposit.	Spectroscopic examination.	Amount of haemoglobin.
1st day	560 c.c.	—	—	Porter coloured			
2nd day (evening)	840 c.c.	—	—	Reddish brown	Slight whitish deposit on centrifugalising, consisting of granular casts and epithelial cells. No red cells.	Oxyhaemoglobin and methaemoglobin bands present.	Chocolate coloured precipitate on acidifying and boiling equalled 0·5 % of haemoglobin. Total amount of haemoglobin passed during the day was 4·2 gr.
3rd day, 8.0 a.m. ,, 9.0 ,,	336 c.c.	1·016	Alkaline	Red	,, ,,	,,	Chocolate coloured precipitate on acidifying and boiling equalled 0·7 % haemoglobin. Total amount of haemoglobin passed during the day was 2·5 gr.
4th day	336 c.c.	1·015	Acid	Amber	Very slight deposit, as above.	No bands	White precipitate on acidifying and boiling equalled $\frac{1}{10}$ column.
5th day	544 c.c.	1·015	,,	,,	,,	,,	,,
6th day	500 c.c.	—	—	,,	,,	,,	,,
7th day	1400 c.c.	—	—	,,	,,	,,	,,
8th day	840 c.c.	—	—	,,	,,	,,	,,
9th day	420 c.c.	—	—	,,	,,	,,	,,

Post-mortem examination.—Body fairly well nourished. Skin sallow. No signs of decomposition. Rigor mortis well marked. No excess of fluid in the pericardial cavity; heart muscle healthy; valves of heart normal; no atheroma of aorta. Pleura free from adhesions, no effusion in pleural cavities. Lungs markedly congested and oedematous, no tubercle present. No fluid in the peritoneal cavity. Liver healthy in aspect. Spleen slightly enlarged. The kidneys were slightly enlarged; capsules stripped readily; cortex not markedly altered in naked eye aspect; pyramids dark, presenting a striated appearance on section, the collecting tubules being easily seen with the naked eye; pelvis of kidney normal. Suprarenals and pancreas normal. Stomach and intestines normal. Bladder, which contained a little amber coloured urine, was normal in appearance. The brain and its membranes presented no macroscopic change.

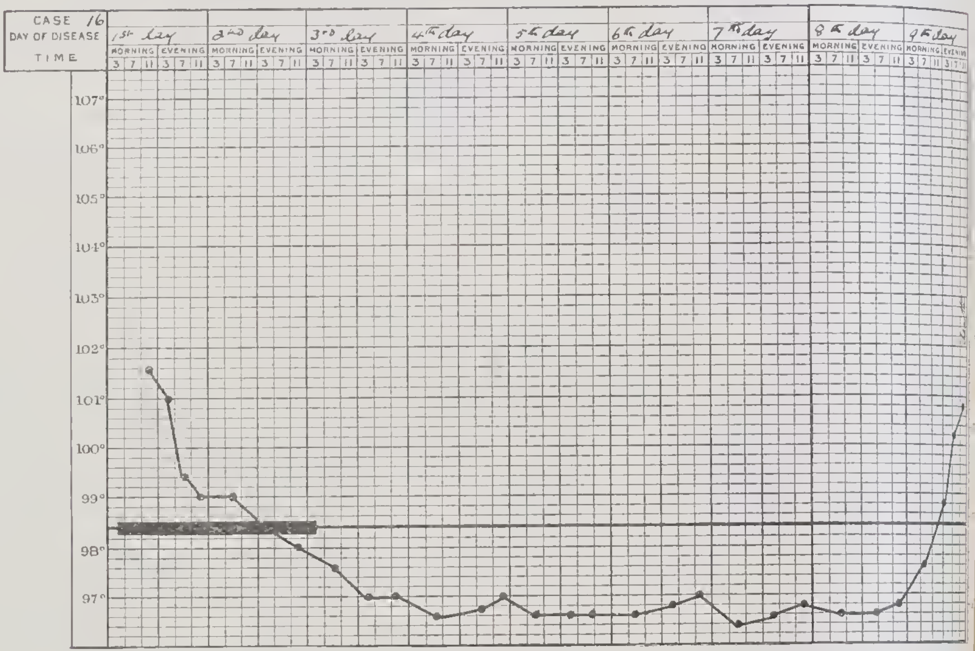

FIG. 81. Blackwater Fever, Case 16. Temperature Chart.

Microscopic examination. The kidneys exhibited in many of the uriniferous and collecting tubules plugs of brownish material made up of granules, 5μ to 2μ or less in diameter, and granular masses, together with epithelial cells and flocculent débris; further details are given on pp. 119-123. The lumen of the uriniferous tubules was wide, but did not exhibit the marked distension seen in the kidneys of Case 7a. The interstitial tissue was free from haemorrhage, and showed no cell infiltration or increase of connective tissue. No cirrhosis or fatty degeneration of the liver or malarial pigment in its vessels. No malarial parasites or pigment was found in the spleen.

BLACKWATER FEVER. CASE 17.

Male, thirty-two years of age. Planter. European.

Has lived in Nyasaland for ten years, during which time he has had one or two attacks of fever every wet season. In the course of the last eight years he has had four attacks of blackwater fever. He only occasionally takes quinine. He suffered from an attack of fever four days ago. T. 105° F. Took twenty grains of quinine. He remained ill for the next three days, and had fifteen grains of quinine each day.

1st day. Was still ill, and took ten grains of quinine in the morning. During the evening he noticed that he was passing blackwater. He was brought into hospital the same evening. His condition was one of considerable exhaustion, with troublesome vomiting. Spleen slightly enlarged, and just palpable. T. 101° F.

2nd day. His condition to-day was unchanged, and the vomiting continued all day. T. 105° F. This rise in temperature was accompanied by a very severe rigor. The urine was porter coloured.

3rd day. The patient was rather better this morning and the temperature almost normal. The urine was still highly coloured.

4th day. The urine became decidedly lighter towards evening.

5th day. Patient feels quite well. Urine almost clear.

6th day. The urine was amber coloured. Patient's subsequent recovery was uninterrupted.

Condition of blood.—1st day. At 11-30 p.m. patient's oxalated plasma was of a deep orange colour, with a distinct red tint, containing 0·65 per cent. of haemoglobin in solution. No malarial parasites and no pigmented leucocytes were found in the blood

2nd day. At 9 a.m. patient's oxalated plasma was of a reddish orange colour, containing 0·67 per cent. of haemoglobin in solution. No malarial parasites and no pigmented leucocytes were found in the blood. At 3-15 p.m. patient's oxalated plasma was of a reddish orange colour, containing 0·95 per cent. of haemoglobin in solution.

3rd day. At 10-30 a.m. patient's oxalated plasma was of a reddish orange colour, containing 0·48 per cent. of haemoglobin in solution. No malarial parasites and no pigmented leucocytes were found in the blood.

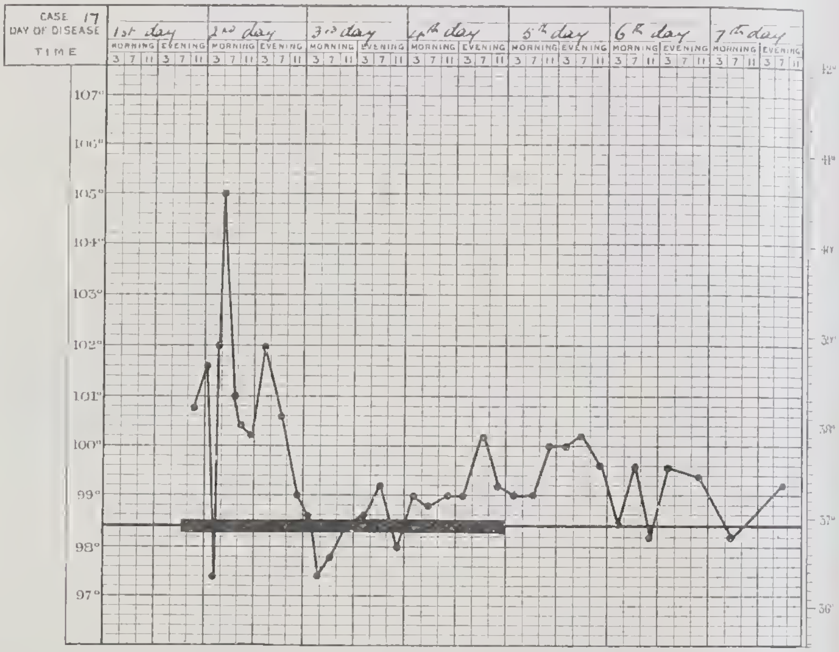

Fig. 82. Blackwater Fever, Case 17. Temperature Chart.

4th day. At 11-45 a.m. patient's oxalated plasma was of a deep orange colour with no red tint, containing 0·18 per cent. of haemoglobin in solution. On examining the blood with the aid of the haemocrit, it was found to contain 22·5 per cent. by volume of red cells. A haemoglobinometer reading of 21·5 divisions of von Fleischl's scale was obtained (equivalent to 0·07 per cent. of wet red cells).

Condition of urine.

Date and time.	Amount.	Sp. gr.	Reaction.	Colour.	Appearance and amount of deposit	Spectroscopic examination.	Amount of haemoglobin.
1st day, 11.0 p.m.	285 c.c.	1·020	Acid	Claret coloured	—	Oxyhaemoglobin and methaemoglobin bands present.	1·45 % (corresponding to 3·6 gr. of haemoglobin). Total haemoglobin passed during the day was 3·6 gr.
2nd day, 12.30 a.m.	28 c.c.	1·015	,,	Dark red coloured	Slight deposit on centrifugalising consisting of granular casts and epithelial cells and red cells.	Oxyhaemoglobin bands present but no methaemoglobin bands.	3 % (corresponding to 0·8 gr of haemoglobin). Chocolate precipitate on acidifying and boiling was 3·5 % of haemoglobin.
,, 4.0 ,,	85·5 c.c.	—	—	,,	,, ,,	,,	1·9 % (corresponding to 1·5 g. of haemoglobin). Chocolate precipitate on acidifying and boiling equalled 3 % of haemoglobin.
,, 4.30 ,,	28 c.c.	—	Alkaline	,,	,, ,,	,,	1·5 % (corresponding to 0·45 g. of haemoglobin). Chocolate precipitate on acidifying and boiling 2·5 g. of haemoglobin.
,, 7.0 ,,	171 c.c.	1·014	,,	,,		Oxyhaemoglobin and methaemoglobin bands present.	1·3 % (corresponding to 2·4 g. of haemoglobin). Chocolate coloured precipitate on acidifying and boiling equalled 1·3 % of haemoglobin.
,, 9.30 ,,	142·5 c.c.	—	,,	Porter coloured	Deposit on centrifugalising about $\frac{1}{25}$th column consisting of granular casts, epithelial cells and casts. No red cells.	,,	1·4 % (corresponding to 1·5 g. of haemoglobin).
,, 12.15 p.m.	142 c.c.	1·014	,,	,,	,, ,,	,,	1·4 % (corresponding to 1·5 g. of haemoglobin). Chocolate precipitate on acidifying and boiling 2·5 % of haemoglobin.

Date and time.	Amount.	Sp. gr.	Reaction.	Colour.	Appearance and amount of deposit.	Spectroscopic examination.	Amount of haemoglobin.
2nd day, 1.45 p.m.	171 c.c.	1·014	Alkaline	Dark red	Deposit on centrifugalising about $\frac{1}{25}$th column consisting of granular casts, epithelial cells and casts. No red cells.	Oxyhaemoglobin and methaemoglobin bands present.	1·5 % (corresponding to 2·5 g. of haemoglobin). Chocolate precipitate on acidifying and boiling 2·5 % of haemoglobin.
,, 3.10 ,,	228 c.c.	1·012	,,	,,	,, ,,	,,	2·0 % (corresponding to 4·5 g. of haemoglobin). Chocolate precipitate on acidifying and boiling equalled 2·5 % of haemoglobin.
,, 5.15 ,,	171 c.c.	1·012	,,	,,	,, ,,		1·6 % (corresponding to 2·7 g. of haemoglobin). Chocolate precipitate on acidifying and boiling equalled 2·5 % of haemoglobin.
							Total haemoglobin passed during the day was 17·9 g. of haemoglobin.
3rd day, 4.15 a.m.	85·5 c.c.	—	,,	Dark reddish brown	,, ,,	,	Chocolate precipitate on acidifying and boiling equalled 2·3 % of haemoglobin.
,, 6.10 ,,	70 c.c.	—	,,	Reddish brown	,, ,,	,,	,,
,, 9.5 ,,	114 c.c.	—	,,	Porter coloured	Deposit on centrifugalising $\frac{1}{12}$ column, consisting of granular casts and epithelial casts, and renal epithelial cells and few red cells.	,,	0·5 % (corresponding to 0·6 g. of haemoglobin). Chocolate precipitate on acidifying and boiling equalled 2·3 % of haemoglobin.
,, 11.20 ,,	57 c.c.	1·014	Acid	,,	,, ,,	,,	0·4 % (corresponding to 0·2 g. of haemoglobin). Chocolate precipitate on acidifying and boiling equalled 2·[illegible] % of haemoglobin.

Date and time.	Amount.	Sp. gr.	Reaction.	Colour.	Appearance and amount of deposit.	Spectroscopic examination.	Amount of haemoglobin
3rd day, 12.30 p.m.	28·5 c.c.	1·012	Acid	Porter coloured	Deposit on centrifugalising $\frac{1}{12}$ column, consisting of granular casts and epithelial casts. and renal epithelial cells and few red cells.	Oxyhaemoglobin and methaemoglobin bands present.	0·3 % (corresponding to 0·1 g of haemoglobin). Chocolate precipitate on acidifying and boiling equalled 2 % of haemoglobin.
,, 1.10 ,,	285 c.c.	1·012	,,	,,	,, ,,	,,	0·25 % (corresponding to 0·7 g. of haemoglobin). Chocolate precipitate on acidifying and boiling equalled 1·5 % of haemoglobin.
,, 3.0 ,,	142 c.c.	1·012	,,	,,	,, ,,	,,	0·36 % (corresponding to 0·7 g. of haemoglobin). Chocolate precipitate on acidifying and boiling equalled 2 % of haemoglobin.
,, 4.0 ,,	114 c.c.	1·011	,,	,,	,, ,,	,,	0·25 % (corresponding to 0·3 g. of haemoglobin). Chocolate precipitate on acidifying and boiling equalled 1·7 % of haemoglobin.
,, 5.0 ,,	256 c.c.	1·010	,,	,,	Deposit on centrifugalising slight, consisting of renal epithelial cells in large numbers and a few granular and epithelial casts.	,,	0·2 % (corresponding to ·5 g. of haemoglobin). Chocolate precipitate on acidifying and boiling equalled 1·7 % of haemoglobin.
,, 6.15 ,,	285 c.c.	1·005	,,	,,	,, ,,	,,	·15 % (corresponding to ·4 g of haemoglobin). Chocolate precipitate on acidifying and boiling equalled 1·2 % of haemoglobin

Date and time.	Amount.	Sp. gr.	Reaction.	Colour.	Appearance and amount of deposit.	Spectroscopic examination.	Amount of haemoglobin.
3rd day, 7.0 p.m.	228 c.c.	1·008	Acid	Porter coloured	Deposit on centrifugalising slight, consisting of renal epithelial cells in large numbers and a few granular and epithelial casts.	Oxyhaemoglobin and methaemoglobin bands present.	0·15 % (corresponding to 0·3 g. of haemoglobin). Chocolate precipitate on acidifying and boiling equalled 1·2 % of haemoglobin.
,, 8.30 ,,	228 c.c.	1·014	,,	Reddish brown	,, ,,	Oxyhaemoglobin bands present, methaemoglobin bands absent.	0·1 % (corresponding to 0·2 g. of haemoglobin). Chocolate precipitate on acidifying and boiling equalled 0·7 % of haemoglobin.
,, 9.30 ,,	114 c.c.	1·014	,,	,,	,, ,,	..	0·1 % (corresponding to 0·1 g. of haemoglobin). Chocolate precipitate on acidifying and boiling equalled 1 % of haemoglobin.
,, 11.45 ,,	200 c.c.	1·010	,,	Brown	,, ,,	..	0·1 % (corresponding to 0·2 g. of haemoglobin). Chocolate precipitate on acidifying and boiling equalled 0·5 % of haemoglobin.
							Total haemoglobin passed during day was 4·2 g.
4th day, 3.0 a.m.	342 c.c.	1·010	,,	,,	,, ,,	..	0·1 % (corresponding to 0·3 g. of haemoglobin). Chocolate precipitate on acidifying and boiling equalled 0·5 % of haemoglobin.
,, 6.15 ,,	314 c.c.	1·009	,,	,,	Deposit on centrifugalising slight, consisting almost entirely of epithelial cells and very few granular casts and red cells.	,,	0·1 % (corresponding to 0·3 g. of haemoglobin). Chocolate precipitate on acidifying and boiling equalled 0·5 % of haemoglobin.

Date and time.	Amount.	Sp. gr.	Reaction.	Colour.	Appearance and amount of deposit.	Spectroscopic examination.	Amount of haemoglobin.
4th day, 9.40 a.m.	400 c.c.	1·009	Acid	Brown	Deposit on centrifugalising slight, consisting almost entirely of epithelial cells and very few granular casts and red cells.	Oxyhaemoglobin bands present, met-haemoglobin bands absent.	0·1 % (corresponding to 0·4 g. of haemoglobin). Chocolate precipitate on acidifying and boiling equalled 0·5 % of haemoglobin.
,, 2.0 p.m.	456 c.c.	1·010	,,	Light brown	,, ,,	,,	0·06 % (corresponding to 0·3 g. of haemoglobin). Light brown precipitate on acidifying and boiling equalled 0·5 % of haemoglobin.
,, 4.30 ,,	285 c.c.	1·009	,,	,,	Deposit on centrifugalising slight, consisting almost entirely of epithelial cells and very few granular casts.	,,	0·07 % (corresponding to 0·2 g. of haemoglobin). Light brown precipitate on acidifying and boiling equalled 0·5 % of haemoglobin.
,, 7.30 ,,	228 c.c.	1·012	,,	Dark amber coloured	,, ,,	,,	0·05 % (corresponding to 0·1 g. of haemoglobin). Light brown precipitate on acidifying and boiling equalled 0·4 % of haemoglobin.
,, 10.0 ,,	170 c.c.	1·012	,,	,,	,, ,,	,,	0·06 % (corresponding to 0·1 g. of haemoglobin). Light brown precipitate on acidifying and boiling equalled 0·4 % of haemoglobin.
,, 11.45 ,,	340 c.c.	1·012	,,	,,	,, ,,	,,	0·05 % (corresponding to 0·17 g. of haemoglobin). Light brown precipitate on acidifying and boiling equalled 0·3 % of haemoglobin. Total haemoglobin passed during the day was 1·8 g.

Date and time.	Amount.	Sp. gr.	Reaction.	Colour.	Appearance and amount of deposit.	Spectroscopic examination.	Amount of haemoglobin.
5th day, 2.0 a.m.	170 c.c.	1·010	Alkaline	Dark amber coloured	Deposit on centrifugalising slight, consisting almost entirely of epithelial cells and very few granular casts.	Oxyhaemoglobin bands present, methaemoglobin bands absent.	0·06 % (corresponding to 0·1 g. of haemoglobin). Light brown precipitate on acidifying and boiling equalled 0·4 % of haemoglobin.
,, 6.30 ,,	430 c.c.	1·010	,,	,,	Deposit on centrifugalising slight, consisting of numerous renal epithelial cells with few granular casts.	,,	0·05 % (corresponding to 0·2 g. of haemoglobin). Light brown precipitate on acidifying and boiling equalled 0·4 % of haemoglobin.
,, 10.45 ,,	228 c.c.	1·009	,,	,,	,, ,,	,,	0·06 % (corresponding to 0·1 g. of haemoglobin). Light brown precipitate on acidifying and boiling equalled 0·4 % of haemoglobin.
,, 12.20 p.m.	256 c.c.	1·010	,,	Brownish	,, ,,	,,	0·1 % (corresponding to 0·25 g. of haemoglobin). Light brown precipitate on acidifying and boiling equalled 0·4 % of haemoglobin.
,, 1.30 ,,	85 c.c.	1·010	,,	Deep amber coloured	,, ,,	,,	Light brown precipitate on acidifying and boiling equalled 0·3 % of haemoglobin.
,, 2.30 ,,	228 c.c.	1·010	,,	,,	,, ,,	,,	,,
,, 4.30 ,,	—	—	—	Amber coloured	No deposit	No bands.	Slight white precipitate on acidifying and boiling.
,, 7.30 ,,	—	—	—	,,	,, ,,	,,	,,
,, 11.0 ,,	—		Acid	,,	,, ,,	,,	No precipitate on acidifying and boiling. Total haemoglobin passed during the day was 0·8 g.

Table 50. Showing the amounts of quinine (given in grains) taken before, during, and shortly after, the attacks of Blackwater fever investigated.

No. of Case	4th	5th	6th	7th	8th	9th	10th	11th	12th	13th	14th	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th	13th	14th	15th	16th	day
1												5 before attack																
2											5	5	6 before attack															
3														5	10	5	5+5+5 before attack											
4											10+5						3+3½	3+3+3	4+4+4	3+3+3	3+3+3	3+3+3	5+5+5	5+5+5	5+5+5 before attack	3+3+3	3+3+3	
5													10	10														
6											10+10 +10																	
6A											10	10																
7													5+5	5+5+5														
7A	10	10	10	10	10	10	15	2½+2½ 2½+2½ 2½+2½	do.	2½+2½ 2½+2½ 2½	2½+2½ 2½+2½ 2½+2½																	
8												15	15	15			3+2½	1+1+1	1+1+1	1+1+1	1+1+1	1+1+1	2+2+2	2+2+2	2+2+2	2+2+2	2+2+2	2+2+2
9											9	9																
10									7½	7½	15	10+5 before attack							2½+1¼	2½+2½	3¾+3¾ +3¾	5+5+5	5+5+5	5+5+5				
11										10		5 before																
12											5	5		1+1+1	2+2+2	3+3+3	4+4+4	5+5+5	5+5+5	5+5+5	5+5+5	5+5+5	5+5+5					
13											4	4 before attack																
14												5	5+5															
14A												10						½	½	1	1	2	2	3	3	5	5	
15									10	5	5	10																
16									5+5+5	5+5+5	5+5+5																	
17								20	15	15	15	10																

Table 52. Synopsis of Cases of Blackwater Fever.

No. of case.	No. of attack.	Day of illness.	Condition of Blood Plasma.			Condition of Red Blood Cells.			Condition of Urine.				
			Colour.	Haemolysin.	Amount of dissolved haemoglobin.	Parasites (about 5000 red cells examined).	Smallest concentration of quinine bi-hydrochloride which caused complete haemolysis in three hours (see Table 24).	Haemoglobin volume.	Colour.	Amount of dissolved haemoglobin.	Red blood cells.	Suspended matter.	Change in quantity.
1	Second attack	1st day							Porter coloured.				
		2nd ,,				None found			Deep red.				
		3rd ,,				,,			..		Present		
		4th ,,				,,			Amber coloured.				
		15th ,,		No isolysin		,,			..				
2	Second attack	1st ,,	Dark orange, no red tint	,,	0·13 %	,,			Yellow.				
		2nd ,,	,, ,,	,, ,,	0·13 % 0·13 %	,, ,,			Chocolate coloured.	No oxyhaemoglobin bands.	,,	Chocolate brown precipitate: granular casts and masses, pus cells and bacilli.	
		3rd ,,	,,	,,	0·13 %	,,			Dark amber coloured.	,,	,,	,, ,,	
		4th ,,				,,			..	,,	Absent	Pus cells and bacilli.	
3	Fifth attack	5th ,,	Dark orange	No autolysin	0·16 %	Present			Yellow.				
		6th ,,	Dark orange with reddish tint ,,	,, ,,	0·57 % 0·40 %	None found	0·045 %	0·91	Port wine red.	1·4 % to 1·3 %	..	Whitish precipitate: granular masses, granular and epithelial casts.	
		7th ,,	,,	,,	0·08 %	,,	0·045 %	0·91	Porter coloured.	1·3 % to 0·1 %	Present	,, ,,	
		8th ,,	Orange	,,	0·08 %	..	0·053 %	1·11	Brown to amber.	Trace	Absent	.. ,,	
		[illegible]			[illegible]	..			Amber coloured.	Absent	..	Precipitate much	

TABLE 52.—(*Continued*). Synopsis of Cases of Blackwater Fever

No. of case.	No. of attack.	Day of illness.	Condition of Blood Plasma.			Condition of Red Blood Cells.			Condition of Urine.				
			Colour.	Haemolysin.	Amount of dissolved haemoglobin.	Parasites (about 5000 red cells examined).	Smallest concentration of quinine bihydrochloride which caused complete haemolysis in three hours (see Table 24).	Haemoglobin volume.	Colour.	Amount of dissolved haemoglobin.	Red blood cells.	Suspended matter.	Change in quantity.
4	Second attack	1st day							Dark red.				
		2nd ,,							,,				
		3rd ,,							Red to amber coloured.				
		4th ,,							Amber coloured.	Absent		No suspended matter.	
		5th ,,				None found			,,	,,		,,	
		6th ,,	Dark orange	No isolysin	0·13 %	,,			Dark amber coloured.	,,		,,	
		7th ,,							Amber coloured.				
		8th ,,							,,	,,		,,	
		14th ,,							Burgundy red.				
5	Second attack	1st ,,							Yellow.				
		2nd ,,							,,				
		3rd ,,							Port wine red.				
		4th ,,							Claret red.				
		5th ,,							Port wine red to reddish amber.	Present		Brown precipitate: granular casts.	
		6th ,,	Orange	No autolysin	0·13 %	,,	About 0·045 % (Compare Table 24)		Brownish amber.	Absent		No suspended matter.	

TABLE 52.—(*Continued*). Synopsis of Cases of Blackwater Fever.

No. of case.	No. of attack.	Day of illness.	CONDITION OF BLOOD PLASMA.			CONDITION OF RED BLOOD CELLS.			CONDITION OF URINE.				
			Colour.	Haemolysin.	Amount of dissolved haemoglobin.	Parasites (about 5000 red cells examined).	Smallest concentration of quinine bihydrochloride which caused complete haemolysis in three hours (see Table 24).	Haemoglobin volume.	Colour.	Amount of dissolved haemoglobin.	Red blood cells.	Suspended matter.	Chan in quantity.
6	Third attack	1st day							Porter coloured.				
		2nd ,,							,,				
		3rd ,,	Dark orange	No isolysin	0·16 %	None found			Faint red to amber coloured.		Present	Granular casts and renal epithelial cells.	
		4th ,,							Amber coloured.			No suspended matter.	
6a	Fourth attack	1st ,,							Porter coloured.				
		2nd ,,							Brown.	No oxyhaemoglobin bands.	,,	Granular casts and renal epithelial cells.	
		3rd ,,	Pale orange	No autolysin	0·16 %	,,			,,	,,	,,	,, ,,	
		4th ,,							,,	,,	,,	,, ,,	
		5th ,,							Amber coloured.	,,	,,	,, ,,	
7	Second attack	1st ,,							,,				
		2nd ,,							,,				
		3rd ,,							,,				
		4th ,,	Dark orange	,,	Less than 0·13 %		About 0·045 % (Compare Table 24)	1·18	Porter coloured.	,,	Absent	Abundant chocolate coloured precipitate : brown granules and granular casts.	
		5th ,,							Dark amber coloured.	,,	,,	,, ,,	
		6th ,,							Amber coloured.		,,	Chocolate coloured precipitate : brown granules and granular casts.	

TABLE 52.—(*Continued.*) Synopsis of Cases of Blackwater Fever.

No. of case.	No. of attack.	Day of illness.	CONDITION OF BLOOD PLASMA.			CONDITION OF RED BLOOD CELLS.			CONDITION OF URINE.				
			Colour.	Haemolysin.	Amount of dissolved haemoglobin.	Parasites (about 5000 red cells examined).	Smallest concentration of quinine bihydrochloride which caused complete haemolysis in three hours (see Table 24).	Haemoglobin volume.	Colour.	Amount of dissolved haemoglobin.	Red blood cells.	Suspended matter.	Change in quantity.
7a	Third attack	1st day	Dark orange with reddish tint	No autolysin	0·74 %	None found	0·053 %		Porter coloured.	0·4 % to 1·2 %	Present	Brownish black deposit : red cells, granular casts.	437 c.c
		2nd ,,							Port wine to yellow.	1·2 % to 0·0 %	,,	,, ..	18 c.c
		3rd ..							Yellow.		..	Slight light yellow deposit : granular casts, renal epithelium.	21 c.c
		4th ..							..		..	.. ..	11 c.c
		5th ..							..		..	.. ..	17 c.c.
		6th ..							..		..	.. ,,	33 c.c.
		7th ..							..		..	.. ..	48 c.c
		8th ..							..		,,	.. ..	41 c.c
		9th ..							..		..	.. ..	23 c.c.
		10th ..							..		.	. .	43 c.c
8	First attack	1st ..							..				
		2nd ..							..				
		3rd ..							..				
		4th ..							Porter coloured.				
		5th ..	Deep orange	,,	0·13 %	..	0·045 %		Deep red to light amber.	·2 %	..	Granular casts, renal epithelial casts.	
		6th ..	,,		0·07 %	..	0·045 %		Amber coloured.		Absent	No suspended matter.	
		7th ..							Red.				
		8th ..							Amber coloured.				